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THE CELL THEORY. II* A MODERN CONCEPT OF THE CELL AS A STRUCTURAL UNIT¹

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YEAR by year, step by step, since Wöhler synthesized urea in the laboratory in 1828 and showed that organic chemistry, the chemistry of life, was merely a more complicated inorganic chemistry; that the difference between the compounds formed in the living organism and those outside the living world was not one of *kind* but merely of *degré of complexity*, the boundary wall between the chemistry of life and non-life has been repeatedly shattered. It is now becoming increasingly apparent that the plan of organization exhibited in the living world, with the cell as the basic unit, does not differ intrinsically from that exhibited by inorganic materials but is a plan which, built upon the relatively simple arrangements of materials outside the protoplasmic sphere, has become amazingly elaborated and of extreme complexity.

The phenomena characteristic of life are possible only when a tremendously complex association of certain elements is present. But the materials used in the protoplasmic mélange—the atoms of carbon, hydrogen, nitrogen, oxygen, sulphur and various other elements—are the

* These four papers conclude the Symposium on the Cell Theory presented at the meeting of the American Association for the Advancement of Science at Richmond, Va., in December, 1938, the first part of which has been published in the November-December, 1939, issue of THE AMERICAN NATURALIST.

¹ Certain portions of the material in this paper have been previously presented in various addresses given at Brown University (April, 1936); Connecticut Academy of Arts and Sciences, New Haven; A.A.A.S., Denver meeting, 1937; Research Laboratory, General Electric Company; Rice Institute, and the University of Alabama.

same in living materials as elsewhere. And, as we all know, by means of the cycles of elements in nature, there is a continuous interchange of these materials from the inorganic to the organic and back to the inorganic. Now every element has its own unchanging characteristics; the structural features and functional behavior of a carbon atom or the atom of any other element is not changed when temporarily built into the protoplasm of a living organism. But the almost incomprehensible complexity of living materials, constructed from a wide assortment of elements, is accompanied by unique phenomena maintained by the utilization of radiant energy—the characteristics of the living state—which are beyond and different from those of the individual elements concerned or from any known association of elements outside the living organism. Even at present the scientist has nothing definite to offer as to when or where or how living phenomena originated, nor as to the basic features of the complex chemistry essential to life.

But turning from the functional to the organizational or structural characteristics of life a great deal of knowledge is now available, based upon contributions from physicists, chemists and biologists, which give a clear view of the elemental patterns of protoplasm: the field of molecular biology. Largely responsible for these advances are the x-ray and the ultracentrifuge of the physicist, the intensive chemical studies on proteins and other organic compounds and the cytogenetic studies of the biologist. It will be profitable to consider some of the data secured from each of these fields in an endeavor to correlate recently established facts relative to the building materials of protoplasm.

THE USE OF THE X-RAY IN THE DETERMINATION OF MOLECULAR PATTERNS

In connection with studies on the nature of x-radiation, the Swiss physicist, Laue, in 1912, suggested the irradiation of inorganic crystalline substances with x-rays in the belief that the distance between the sheets of atoms mak-

ing up the internal space lattice pattern of the crystal were of the same order of magnitude as the wave-lengths of the x-rays, which are about $1/10,000$ that of light waves. If such were the case, the x-rays would be diffracted in accordance with the particular crystal pattern, just as light rays are diffracted by ruled diffraction gratings. This suggestion of Laue proved to be correct, and marked the beginning of the x-ray technique which, as developed and elaborated by the Braggs and others, soon became the most searching of all known tests for crystallinity. It may be said, in the words of Astbury, to be a "scientific substitute for human vision to compensate us for the fact that the only ether waves are some 10,000 times too coarse to show up the shape of the molecules." The use of x-rays to determine the basic molecular patterns of inorganic materials during the past quarter of a century has yielded results of the highest scientific and economic importance and, in fact, revolutionized our knowledge of the basic structure of important metals and minerals.

The structure of any crystalline substance as revealed by the x-ray is essentially uniform, that is, there is a definite characteristic unit pattern formed by the constituent atoms and molecules which is continuously repeated throughout the substance; a pattern which, though ultra-microscopic, may be determined by the proper treatment of the data obtained from the diffraction of the x-rays recorded on a photographic plate. This condition may be visualized by comparing the pattern in a crystalline substance with the visible repeat pattern in wall paper or a woven textile. Any regular arrangement of the molecules in a substance constitutes a crystal and will diffract x-rays in a definite pattern which is continuously repeated and can be identified. This repeat pattern in a crystalline substance is the "unit-cell, that is, the size of the smallest area containing the unit of atomic or molecular pattern which is repeated from end to end of the crystal."

The molecule of the chemist is usually defined "as the smallest portion of an element or compound that retains chemical identity with the substance in mass." As such,

the molecular unit of inorganic substances or even that of a much more complex organic substance is far below microscopic visibility. But x-ray studies first revealed the basic fact that the molecules do not exist as independent units in the solid or crystalline substances. The constituent atoms are all bound together to form the larger units of the crystal pattern without molecular separation. Thus to take the relatively simple example of sodium chloride: the data obtained from the x-ray plates show that in the crystal pattern present throughout this material each atom of sodium is surrounded by six atoms of chlorine, and each atom of the latter in turn is similarly surrounded by six atoms of sodium. Sodium chloride molecules, each with one atom of sodium and one of chlorine, do not exist as independent units.

The basic implications of such results from the x-ray analyses of crystals were very early recognized by Langmuir. In his now classic contribution on "The Constitution and Fundamental Properties of Solids and Liquids," published in 1916 and 1917, he stated that up until the time of the work of the Braggs on sodium chloride "it had been taken for granted that crystals were built up of molecules. But from this work . . . it is clear that in crystals of this type the identity of the molecules is wholly lost, except in so far as we may look upon the whole crystal as composing a *single molecule*."

It should be recognized that the association of the independent molecules in the development of a characteristic crystal pattern occurs only in solids. In a gas the unit molecules are entirely independent of each other except for chance contacts in their rapid movements. Molecules in a liquid are more closely associated than in a gas but even so are not bound together in a rigid crystalline pattern; they are, in general, free to move in any direction and accordingly are uniformly dispersed in a solution.

Apparently the first attempt to apply the revolutionary x-ray technique, which had proved to be so very successful in the inorganic realm, to the more complex organic compounds associated with the living organism occurred in

1921 when Polyani turned the x-ray beam upon cellulose and secured diffraction patterns which revealed the crystalline nature of this most abundant of all plant materials, and his results have been abundantly confirmed and extended. Chemists had long recognized the unit molecule of cellulose as a glucose residue, $C_6H_{10}O_5$, and also that many of these glucose residues were attached as $(C_6H_{10}O_5)_n$. The x-ray studies, by Sponsler, Clark and others, showed that the crystal pattern arose from the linkage of a large number of glucose residue molecules to form a chain. The glucose residue chains, in turn, are held together in parallel arrangement by unknown secondary valences to form a two-dimensional pattern.

The unit cell of this pattern, which is repeated uniformly throughout cellulose, measures $8.35 \text{ \AA.U.} \times 10.3 \text{ \AA.U.} \times 7.9 \text{ \AA.U.}$ and contains four glucose residues. In the micelle of cellulose, as shown by Clark, there are 6,000 glucose residues, or 1,500 unit cells, formed by an aggregation of 60 chains, each containing 100 residues. A cellulose fiber, as in cotton, consists of a great many cellulose micelles or crystals grouped spirally around a longitudinal fiber axis. In the walls of plant cells, however, it has been shown that particles of cellulose, reaching the lower limits of microscopic vision, are formed by an aggregation of 14×10^3 micelles. Seifriz, working with the Spierer lens, has observed that these rod-shaped units, or supermicellae, are arranged end to end to form long plates of cellulose.

Increasingly during the last few years x-rays and polarized light have been used to study the ultramicroscopic structure of various organic materials, particularly the proteins. It now begins to look as if the situation in the study of these protoplasmic building materials may be compared to that in the physical realm twenty-five years ago when the results from the early x-ray studies were first considered. And the results already obtained are of primary importance not only to the workers in the physical and biological sciences but to the economic world as well. Thus far the most extensive researches in the structure of the organic substances by x-rays have been made by the

textile industry in the studies of cotton, wool, silk and other fibers essential to this great industry. Of primary importance in this field are the contributions of Astbury, of the University of Leeds, England, but the data obtained by various other investigators, both in this country and abroad, have also added much that is of first importance.

Comparatively few of the almost infinite number of proteins present on every hand have as yet been subjected to the x-ray technique, but those that have been extensively studied, such as keratin of hair and fibroin of silk, show conclusively that proteins are not amorphous substances, with a "hit and miss" arrangement of the constituent materials, but that they are crystalline with a definite pattern, which, though much more complex than the carbohydrate cellulose and therefore exceedingly difficult to establish, is nevertheless decipherable by the skilled research worker in this field. Just as cellulose is formed by the linkage of glucose residues, so the proteins are constructed by a precise assemblage of amino-acid residues held together by the peptide linkage ($-\text{CO}-\text{NH}-$) to form the so-called polypeptide chains. Recent contributions by Langmuir, Wrinch and others offer evidence that the union of the amino-acid residues may not be in the form of long chains, as in the glucose residues of cellulose, but through a so-called cyclol grouping. This, however, makes no difference for our present consideration since, in either case, it is established that the proteins are crystalline substances.

It is also clearly established that not only are the non-living organic materials, such as cellulose and keratin, crystalline in nature, but protoplasm itself, present in various types of highly differentiated cells as, for example, those of muscle and nerve, has a rigid structural plan extending from molecular levels to visible cell units crystalline in nature. And even before the recent revelations of the x-rays, the geneticists, headed by Morgan, had established the definite linear pattern of the particulate hereditary units or genes in the chromosomes as a result of their controlled breeding experiments and also by the production of mutations by altering the gene pattern in the germ

cells through the use of x-rays (Muller)—a very different use of this important scientific tool than in the determination of the crystalline patterns by diffraction.

The recent studies of Painter on the giant salivary gland chromosomes and the determination thereby of their behavior in synaptic phenomena have given visual confirmation to the theories proposed on the basis of the breeding experiments. Apparently the actual stainable material of a gene is beyond the resolving power of the microscope, though some authorities are convinced that in certain instances the genes may actually be seen. At all events, it is definitely established that the genes in a chromosome are arranged in a precise pattern. Even if the x-ray had never thrown any light on protein structure, data from genetical and cytological studies would have revealed the crystalline nature of the chromosomes. Each chromosome may be regarded on this basis, as recognized by Bridges and others, as a huge protein molecule or crystal in which the genes and the accompanying substances are built into a characteristic pattern.

Thus the present situation relative to the structural pattern of the life-materials may be summarized by saying that, in essentially all instances where the new methods have been used, evidence has been obtained of a basic crystalline pattern as in inorganic solids. It therefore becomes evident once more that the difference between the inorganic and the organic lies primarily in the much greater complexity of the latter and not in a different method of construction. Many major details of the crystalline patterns of the varied lifestuffs are still undisclosed, but the foundations have been laid, and the next few years will undoubtedly record substantial progress.

The results that have been obtained showing crystallinity as a characteristic of all solids, inorganic or organic, represent another definite breach in the wall which has long separated form and structure in the biological world from inorganic organization. To the biologist who has been taught and who has then taught others that protoplasm is essentially amorphous—a sort of syrupy liquid

which flows hither and yon, or that "protoplasm is an aqueous solution in which are suspended colloidal substances of great complexity"—the results establishing the fact that the proteins, the building stuffs of life, are crystalline, just as are the inorganic solids, come as information of the highest importance, pregnant with possibilities for future major advances in biological knowledge.

MOLECULAR SIZE IN THE PROTEINS AND VIRUSES

In the previous paragraphs, attention has been given to the determination of the crystalline patterns characteristic of solids in the inorganic and organic worlds; patterns in which the individual molecules are merged. But our knowledge of the building materials of life has also been greatly increased by the study of protein molecules present in solution where the individual molecules can be studied and important characteristics determined. In this connection information relative to molecular size and orientation is of particular importance for the present discussion. The orientation of molecules may be studied in surface films formed by contact between two liquids, as seen, for example, in a film of oil on water. Molecular behavior and orientation in surface films were studied by Hardy some twenty-five years ago in connection with problems of lubrication. The experiments in later years by Harkins, Langmuir and their associates have proven very fruitful in various fields but particularly in giving accurate data of molecular size as determined by the use of monomolecular films. The very recent development of the multi-layered films of various organic compounds, including proteins, at Langmuir's laboratory has given additional results of the utmost importance to the biologist in showing just how the membranes of basic importance in biological systems are formed from repeated oriented layers of monomolecular films.

There are two other reliable sources of information, which have become available in the last few years, for the determination of molecular size. The first of these, the

ultracentrifuge, permits the determination of the size of molecules or molecular particles in solution by measuring their rate of sedimentation in response to a tremendous centrifugal force, which may be several million times the force of gravity. Finally, the invention and use of colloidal filtration membranes with a wide range of accurately graded pore sizes have yielded very important results.

The data accumulated in the last few years from these sources have made it possible to determine the molecular weights and dimensions of a wide range of proteins and viruses for comparison with cellular units. Stanley has tabulated the results of various workers and constructed an interesting and important chart to present them graphically. The data thus presented show a gradual increase in the unit size, beginning with the ultramicroscopic protein molecules and continuing with the virus and bacteriophage units to the cell of the smallest known bacterial organism, and, finally, to cellular units of microscopic visibility. Furthermore, at the two extremes of the series there is an overlapping by the virus units. Thus the smallest known virus particle, with a diameter of 10 $m\mu$, is considerably smaller than some of the largest known protein molecules as found, for example, in hemocyanin, the molluscan respiratory pigment. At the other end of the series is the comparatively enormous unit of the Psittacosis virus with a diameter of 275 $m\mu$, which is almost twice that of the smallest known cell, the pleuropneumonia organism, with a diameter of 150 $m\mu$. Comparing the Psittacosis virus with larger cell types, shows it to be about one third the diameter of *Bacillus prodigiosus* (750 $m\mu$) which, in turn, measures one tenth the diameter of the normal human red blood corpuscle (7.5 μ).

The dimensions of a considerable number of species of viruses and bacteriophages, lying between these two extremes, have been established: The data presented in the chart show that "the viruses form an unbroken series with respect to size from protein molecules to bacteria, but at either end . . . there is an overlapping. Certain viruses

are smaller than accepted protein molecules, and others are larger than accepted bacteria. It is obvious that on the basis of filterability it is impossible to draw lines that sharply divide the viruses from bacteria or from protein molecules."

Furthermore, it has been shown that the molecules of a virus protein may unite to form crystals which are visible under the microscope. Thus Stanley shows that the molecular units of tobacco mosaic virus protein are aggregated during crystallization to form definite needle-like crystals with a length of about 300 m μ . X-ray measurements of the molecular aggregates of virus proteins have been made by Bawden and Pirie (1937). They find that there "is a regular arrangement of the groups along each particle, and the sharpness of the reflections shows that these particles are sufficiently long for a large number of repeat units to be found in each. Each particle has an internal regularity of the type sometimes found in large molecules, but with the viruses it is on an unusually large scale. In this sense the particle resembles an animal or plant fiber and may be spoken of as crystalline."

Another well-known example of protein crystallization is beautifully shown in the formation of fibrin crystals in blood plasma. Observed under the dark field microscope the uncoagulated blood plasma shows an entirely dark field. Very soon, however, as the crystallization processes proceed, the molecular aggregates increase in size and attain microscopic visibility, the field under observation becoming lighter and lighter until it is soon flooded with a brilliant glow as the fully formed filamentous fibrin crystals fill the field. It has been shown that under the proper experimental conditions further fusion of the fibrin crystals will form fibrils many micra in length. Another significant and interesting example of protein crystallization was reported by Nageotte in 1927 in the case of the protein, collagen, which is the primary constituent of the fibrous connective tissues of the vertebrate body. A solution of collagen may be obtained by placing a tendon for

a time in a weak solution of acetic acid. At a pH of about 4.7 the collagen molecules in the solution of collagen will crystallize to form visible crystals of characteristic shape.

THE CELL AS A STRUCTURAL UNIT

Turning our attention to the cell of the biologist, it is important to inquire whether the newly established facts of organic structure, as just outlined, give any grounds for bringing cellular organization, which is almost universally associated with the living organism, into direct relationship with the structural patterns of the elemental materials from which it is built. In other words, is the cell considered purely as a structural unit an entirely distinct and unrelated entity, or does it represent the climax of a gradually increasing complexity in pattern possessing direct continuity with structural forms of less complexity associated with the materials of the non-living world? May it not be possible that the cell is essentially a protoplasmic crystal in which an almost infinite number of protein molecules, beginning with the genes in the chromosomes, are associated in a definite ultramicroscopic pattern characteristic of the particular type of cell? In such a condition, just as Langmuir early recognized, individual protein molecules are not present, but all are united to form the perfect unit, the complete crystalline pattern of a specific type of protoplasm, the terms molecule, crystal and cell becoming synonymous.

Possibly in a very real sense, therefore, the cell is to be regarded as a molecule of protoplasm; the least amount of this life stuff which will exhibit the characteristics of the living state, just as a single molecule of sugar or hemoglobin or virus protein is the indivisible unit of these substances. This condition becomes particularly evident in the enormous protein molecules of the viruses and the bacteriophage which have the greatest molecular weight of any known protein. Thus the homogeneous unstable nucleoprotein isolated from the phage and reported by Northrop, 1938, has a sedimentation constant corresponding to a molecular weight of about 300 millions. Stanley

has shown that the tobacco virus protein is infective in dilutions of one part to ten billions. Thus it seems probable that a single molecule of virus protein may propagate and cause infection of host tissue as would a cell of a unicellular organism.

There is a sharp divergence of opinion among the biochemists as to whether the unit particles of the viruses and the bacteriophages are merely enormous protein molecules equipped in some way for propagation by autocatalytic reactions or whether they are elementary living agents. Northrop, from his recent studies on the bacteriophage (1938) takes the position that the so-called "living reactions" of the phage units can be "more simply explained by analogy with the autocatalytic formation of pepsin and trypsin than by the far more complicated system of living organisms." He says:

It is extremely improbable that any protein can be synthesized by a purely catalytic reaction under biological conditions since it is known that under such conditions of temperature and pH, proteins hydrolyze slowly into their constituent amino acids and this process may be accelerated by a number of catalysts. If, now, another catalyst could be found which would cause the synthesis of proteins from amino acids without the expenditure of energy, a perpetual motion would result. There is good reason to believe, therefore, that the synthesis of protein requires energy and that it can only take place in a system organized to supply this energy, as in a living cell. Thus the cells of the gastric mucosa synthesize the inert protein, pepsinogen, which is transformed to the active enzyme by an autocatalytic reaction.

A similar mechanism will evidently account for the increase of bacteriophage and other viruses in the presence of living cells. The cells synthesize a "normal" inactive protein. When the active virus of bacteriophage is added, this inactive protein or "prophage" is transformed by an autocatalytic reaction into more active phage. This mechanism accounts for the fact that phage is produced rapidly only in the presence of growing cells, since only in growing cells is synthesis taking place. . . .

It appears to the writer that the assumption that the living host cells synthesize an inert "normal" protein which is changed to the active phage by an autocatalytic reaction accounts for the observed facts as well as does the far more complicated series of assumptions involved in the hypothesis that the phage itself is a living organism.

The opinion of Gortner² is quite the opposite. He states that additional data are necessary before one can eliminate finally the idea

² Reprinted by permission from "Outlines of Biochemistry," by Gortner. Published by John Wiley and Sons, Inc. Second edition, page 458.

of a living organism. If they are "autocatalytic" proteins with the peculiar property of inducing, when injected into living organisms, the production of more proteins of the same type, then they are certainly very unique materials and bridge the gap between living and non-living matter. Autocatalysis, so far as the author is aware, is usually, if not exclusively, a breaking down process, possibly a chain reaction mechanism where the energy for the reaction is passed on from molecule to molecule, once the chain has been set in motion. A building-up autocatalysis, whereby energy is stored and accumulated, is a special type of autocatalysis which involves such a radical revolution of ideas that the evidence in its favor should be very carefully scrutinized before final acceptance. The fact that virus proteins sediment with uniform velocity and that the virus activity of the preparation is unchanged after sedimentation is not necessarily evidence of homogeneity. . . .

Furthermore,

the crystallization of the virus protein may be due to a "polarity" of a living organism, since all cells and organisms have definite electrical polarities. Many colonies of the lower organisms assume characteristic shapes and forms, although the colony is composed of a great number of individual organisms. Although the weight of evidence appears to be at the present moment in favor of the virus proteins being non-living biochemical entities or systems, . . . one should not ignore the possibility that special types of living organisms may be involved in the phenomena. . . . As has already been indicated, if these units are proteins, the gap between the living and the non-living has been almost bridged.

Stanley, from his extensive studies on the viruses, has taken a middle position which, nevertheless, favors the view that they are living agents, though possibly of a retrograde or parasitic nature. Apparently the evidence at hand does not afford indisputable proof that the virus units are either living agents or non-living proteins. However, he feels that the evidence is very decisive that the functional characteristics of the tobacco mosaic are "part and parcel" of the virus protein molecule. They reproduce, they adapt themselves to certain variations in different types of living cells, exhibit "heritable" permanent changes or mutations, and they are destroyed by certain conditions injurious to living cells. Stanley has also shown that the tobacco mosaic virus fulfils Koch's postulates for a disease-producing parasite, except that the purity of the virus materials has to be established by rigid chemical methods rather than by biological tests. His view-point is summarized in the statement (1937) that:

As we go from the admittedly non-living to the admittedly living, I think there must be a transition stage where there are entities that may possess some properties that are considered characteristic of non-living things. What could fill this place more logically than the high molecular weight virus proteins that are intermediate in complexity between the protein enzymes and hormones, the wonderful properties of which we all recognize, and the system of proteins that we call protoplasm and that constitutes life. There is evidence that even within the virus group there is a gradual increase in complexity of structure from the small nucleoproteins to the more elaborate elementary-body type of virus. There is, however, no sharp break despite the fact that the structure of the latter may resemble that of a cell-type organism as much as it resembles that of the smaller viruses. I consider it unimportant whether we call the virus proteins molecules or organisms . . . I have referred to them as molecules solely because of the accident of my training as a chemist.

Possibly the condition may be described by the statement that the viruses are protein molecules, but they are of a size and inherent complexity which permit the establishment of certain functions hitherto known to exist only in living organisms at the cellular level. Just as the virus units have not attained the structural complexity present in cellular units, even so certain of the functional features associated with metabolic activities at cellular levels are largely lacking. But it is evidently of the greatest significance that the building materials of the viruses and of the bacteriophages, as shown by various investigators, are nucleoproteins which are also characteristic of the chromatin and gene complex in the cell nucleus. I recently heard a ranking biologist define a cell "as the immediate sphere of influence of a gene complex." The virus molecule may well be essentially an elementary type of gene complex with no intrinsic "sphere of influence" or cytoplasm surrounding it.

Gortner refers to the experiments of Beams and King in which *Ascaris* eggs were subjected to a force of 400,000_g and nevertheless maintained a normal oxygen consumption and development. From these experiments they concluded that either *Ascaris* protoplasm does not behave as Svedberg's protein systems or else "that spatial relationships in protoplasm are not essential to life processes."

³ Gortner, *loc. cit.*

Gortner states³ that "if no microscope had ever been developed which was powerful enough to render the *Ascaris* eggs visible, one could easily have concluded from these experiments that the *Ascaris* eggs sedimented as uniform-sized protein micelles having an enormous particle weight and that the vital activities characteristic of *Ascaris* eggs were properties of this massive protein 'molecule,' for the *Ascaris* egg would give the usual protein tests and they would appear as 'globular' proteins."

Microscope or no microscope, the possibility is apparent that the description of the *Ascaris* egg as a massive protein molecule with "an enormous particle weight" is correct; that the egg or any other cell is essentially an infinitely elaborate protein complex in which all the protein molecules are built into a characteristic crystalline pattern on the same basic plan as the amino-acid molecules are joined to form the various proteins. As Schmitt recently stated⁴ relative to the existence of a submicroscopic lattice or framework of oriented protein molecules in the cell:

This idea is an outgrowth of modern theories of protein fine structure and is a revival in a more enlightened form of the ancient idea that the ground substance may contain an organized complex of giant protein molecules and that important cell functions may be subserved by this submicroscopic cytoplasmic framework. Experimental embryologists have even suggested that . . . it may form the physical basis for the determination of polarity in cells and of axes of symmetry in the developing embryo. . . . An oriented lattice or cytoskeleton has been demonstrated in one type of cell—the neuron. Neurofibril formation results from the deposition upon this pre-existing lattice of protein material from the axis cylinder.

Thus, for example, the studies of Conklin on the protoplasm of *Crepidula*, in which he showed that the eggs consist of a fluid portion and also of a more resistant viscid portion, the basic spongioplasm, harmonizes with the more recent results.

It has, indeed, been extraordinarily difficult for the cytologist to get a clear vision of the cell as a structural unit because he has been blinded by the brilliance of its functional features and also because he has been unable to

⁴ Indianapolis meeting, 1937, American Society of Zoologists.

penetrate through the ebb and flow of the heterogeneous mixture of complex organic substances which are necessarily accumulated within the cell boundaries to keep the "wheels of life turning." As Seifriz points out, the behavior and appearance of protoplasm indicates, superficially, that it is an emulsion, but this is illusory—there is an underlying ultramicroscopic substance, protoplasm itself, now revealed by the x-ray and other experimental methods, which is continuous and highly organized. All the protoplasmic phenomena have their source in this ultramicroscopic organization from the complex chemistry of life to structural formations of incredible beauty and exactness, from the diatom shell to identical twins. Furthermore, the cell, periodically undergoing mitosis, reveals a temporary crystallinity at the levels of microscopic visibility with both nuclear and cytoplasmic elements united in the complete pattern. The protoplasm in a cell in the intermitic periods is not less organized; the materials are only woven into an ultramicroscopic pattern of different design. The proteins of the cell protoplasm, essential to the maintenance of life phenomena, form a perfect whole; there are no separate molecules; all are joined in a microscopically visible macromolecule, the cell.⁵

In the unicellular organisms a single "cell-molecule" constitutes the complete living unit. Each is as independent as the molecules of a gas floating through currents of air. Far different, however, is the condition in the multicellular organism where differentiation and cellular specialization hold sway. Just as it is impossible for the genes or the chromosomes or the nucleus or the cytoplasm to remain alive outside the cell complex, so it is impossible for the differentiated cells to maintain themselves outside the organism. The differentiated cells are no longer independent units, but they are bound together in the complete organism. Is it merely a superficial analogy to say that the "cell-molecules" associated in the multicellular organism are only repeating the condition of the protein mole-

⁵ Cf. Warren H. Lewis, *Science*, 89: 2314, 400.

cules which are bound together in the protoplasm of each cell to make the functional cellular unit?

The geologists long held to the Catastrophic Doctrine, the primary assumption of which was that "natural forces were more active and powerful in the past geological ages than they are now; that great convulsions of nature had riven the crust asunder into valleys and elevated other portions into mountains." But by the middle of the last century the Uniformitarian School gained the ascendancy, largely through the influence of Hutton and Lyell. On this view it was held that the geological processes had never differed intrinsically from those of the present day. And why should it ever have been thought otherwise, we now ask, since the same primary forces and elemental materials were at work? Is not biology at present, near the middle of the twentieth century, in much the same position as was geology a hundred years ago in failing to observe that there is a Uniformitarian Principle pervading all organization in the inorganic and in the organic as well? The basis of separation between life and non-life is dependent on the *degree of complexity* and not on a difference in *kind*, since the same materials are used in both domains, and they must conform to the same elemental patterns. From the simplest substance in the inorganic world to the most complex patterns of living substance there must be a graded series. Should the processes of evolution begin at the level of the living organism? Were not the union of hydrogen and oxygen to form water; the union of carbon and oxygen to form carbon dioxide; the union of water and carbon dioxide to form sugar; the addition of other elements to the glucose molecule to form protein; were not all these merely stages in the evolutionary processes which have led to ever-increasing complexity and have reached their climax in the world of life?

Needham, in his thought-provoking volume, "Order and Life," quotes Sapper as follows:

We now stand before a problem . . . namely, how is the origin of pattern in material objects in general and in living things in particular to be ex-

plained? Is it not indeed inconceivable that properties should be found in the material complex, which are not the result of the summation of the properties of the components? Are we really forced to the assumption of some supra-material, hyper-individual factors, in order to account for the appearance of the qualitatively new in the organized patterns? In my view there is only one way to picture the organization of the material complex without having recourse to such assumptions; and that is to assume that the qualitatively new in the pattern derives from the properties of the elements involved, but that certain of these properties can only come into operation in connection with certain stages of complexity. There is of course no proof available for demonstrating the rightness of this viewpoint. But it will not be denied that it describes the facts in the simplest way and has the advantage of agreeing with the analogy from the social life of man. . . . If one disagrees with it, one has the choice, *either* of seeking to contest the facts of the existence of additive properties in complex patterns, *or* of regarding them as fundamentally inexplicable and unintelligible.

Nevertheless, there is the possibility that a living organism is something more than the sum of its parts. Such being the case, it is not to be expected that "continual fragmentation will of itself necessarily reveal the true inner meaning of life processes." Since the establishment of the cell theory, living phenomena have been centered in the cell, but now it is apparent that the virus and bacteriophage particles, far below the realm of cellular organization, exhibit certain of the living phenomena characteristic of the cellular level. Accordingly, one may hold that in the intramolecular organization of the virus protein molecule with its "chemical combination of nucleic acid and protein of unusually high molecular weight we have sufficient organization within a single molecule to endow it with the lifelike properties that characterize it." Can it be established that the virus protein molecule is the ultimate and indivisible unit of life? Is there not the possibility that there is something deeper and more fundamental than cells, molecules or even atoms that contains the key to the mystery of life, some as yet undiscovered common factor which underlies all living phenomena and which, in its effect, might be compared, for instance, with the molecular theory which has brought the diverse phenomena associated with the solid, liquid and gaseous states of matter into a unified whole?⁶

⁶ Cf. Heyl, "The Lingering Dryad."

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THE PRESENT STATUS OF MITOSIS

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THE subject of mitosis is one which perhaps should not be mentioned too loudly in the celebration of the names of Schleiden and Schwann. Both men, and Schwann in particular, seem to have carried in their minds the idea that new cells arise by a sort of precipitation or crystallization. In Schleiden's formulation of the idea, a granule (the nucleolus) is differentiated out in the cytoplasm of an already existing cell. A membrane is here precipitated and lifted off by the intrusion of liquid between the two. This represents the nucleus around which, in turn, the cytoplasm is formed in a similar manner. New cells were thus thought to be formed on the inside of older cells. Schwann accepted this idea but suggested that cells may also be formed in the structureless substance outside of a mother cell in what can be described as nothing but a process of organic crystallization.

This conception of the reproduction of cells was recognized as false within a very few years, and by 1846 the work of the botanists Mol and Nüggeli seems to have definitely established that cells arise by some kind of division of other cells. Among zoologists recognition of this fact was long delayed, and it was not until 1858 that the work of Kölliker, Remak and others culminated in the uncompromising position of Virchow that all cells arise by the division of preexisting cells.

Almost as soon as this generalization became accepted, the mechanism of the process of reproduction became the subject of investigation and hypothesis. I shall make no attempt here to trace the discovery of facts or the development of ideas bearing on the question from those days to the present, but the alternating periods of satisfaction and disillusionment have irresistibly brought us to the realization that the process of mitosis is a very complicated one. It is therefore a distinct advantage in our attack on the

problem that for working purposes the division of cytoplasm can be held more or less independent of the division of the nuclear elements. I need not give here in detail the justification for this statement, but certainly there are cases in which the cytoplasm may divide without involving the contained nucleus or, indeed, in the entire absence of a nucleus. In still others the chromosomes may undergo mitosis without an accompanying division of the cytoplasm.

In view of the technical difficulties of analyzing the division process, it is perhaps not surprising that in hardly any other field have workers sought their solutions in analogies to such an extent as here and, it may be added, that in few fields have analogies caused so much trouble.

CYTOPLASMIC DIVISION

The survey of our knowledge concerning the division of the cytoplasm justifies an attitude of optimism. Even though most cells are wretchedly small, the egg cells of some species furnish somewhat better material, it being possible to subject them to experiment under controlled conditions. It is therefore not surprising that we have some rather well-established findings at our disposal which entitle us to prophesy a final solution in the not too distant future. Thus we know from the work of such men as Bütschli, McClendon and Spek that there are definite diffusion currents that intimately involve a redistribution of the surface layers of the egg (the hyaloplasm). Ingenious analogies have been presented which show that alterations in surface tension set up diffusion currents which may bring about division in a drop of homogeneous liquid.

Again, the work of Heilbrunn and Chambers has established that there is a definite cycle of viscosity changes in the course of a mitosis. In this connection Gray has shown that an increase in the extent of two viscous centers may bring about the division of a homogeneous drop that contains them.

Either action may be, and probably often is, linked with

the formation and action of asters and it somehow seems that a drawing together of our information should suggest at least the general outline of an answer to the question. The difficulties that are still preventing this arise from various sources. Thus, as has been stated, nearly all experimental work has been on eggs which, in some respects, offer rather specialized conditions. Again, the analogies that I have mentioned always utilize homogeneous substances such as are certainly not found in the living cell. But all in all it seems to me that we are not far away from a preliminary solution of the problem, even though many details will undoubtedly cause further difficulty.

DIVISION OF THE NUCLEUS

But, when it comes to the analysis of nuclear division or, to state it more exactly, the mitotic movement of chromosomes, no such optimism seems justified. This attitude is in striking contrast to that of the 1880's or even the early 1900's, when even a casual study of the amphister suggested strongly that the motion of the chromosomes to opposite poles must somehow depend on the activity of the fibers that attach to the chromosomes on the one hand and to the centriole on the other. The simplest and most obvious interpretation was that these fibers, anchored at the center, shorten or contract and thus pull the chromosome to the pole. An alternative hypothesis was that the interzonal connections expand so as to push the chromosomes away from each other toward the poles. However, doubts soon arose as closer analysis showed that the fibers could hardly be considered as simple elastic strands for, though doubtless they shorten, they never seem to thicken in the process.¹ And for the second hypothesis it was pointed out that if a pushing action is given by an expansion of the interzonals, an additional force must be involved that guides the chromosomes toward a focal point

¹ Professor Conklin informs me that he is convinced that in at least some forms such thickening nevertheless does occur.

at the pole. There were additional complications that lay in the position taken by chromosomes on the equatorial plate; the question of how fibers growing out from one center met other fibers coming from the opposite center to make continuous or primary fibers; how still other such fibers managed to make connections with the chromosomes, and so on and so on.

Things became so complicated and involved that investigators who were looking for a simple, comprehensive explanation for observed phenomena were seized with a kind of despair which in turn resulted in a tacit agreement that the matter had better be laid on the shelf until advances in other fields might suggest a new attack from another angle. It was this attitude that prepared the ground for the advent of the so-called dynamic hypothesis of the early 1900's.

The obvious resemblance of the amphiasster to an electrostatic field of force had been noted often enough, but it remained for Hartog, Gallardo and R. Lillie to construct working hypotheses on this basis. These men reasoned that if the two centers carry opposite charges the conditions that bring about the resemblance to an electrostatic field are present. But this was too good to be true, and almost at once very valid objections arose. The configuration of amphiastral rays did not really conform to that of the lines of force postulated, for they frequently crossed and anastomosed. Again, the existence of tripolar and quadripolar figures offered the difficulty of amphiastral figures between poles of like sign. Multiple asters always repelled each other; that is, they always seemed to carry the same charge. Hartog's and Gallardo's efforts to escape from these difficulties are marvels of ingeniousness, but the fact remains that the various objections could not be overcome without an extensive adoption of additional and subsidiary assumptions. And, when everything was said and done, such constructions were usually of little aid in regard to our present question, for they involved the chromosomes only in the rôle of minor agents, if at all.

It was left for R. Lillie to round out the picture. There is not time to elaborate on what was in the end a skilfully constructed hypothesis. In its merest outline it postulated that the centers are of like sign, as mutually repelling asters indicated, and that the chromosomes take an equatorial position because they carry a charge of the same sign as the centers and are thus repelled from the opposite points toward a mid position. They are kept there because this middle region of the spindle carries a plus charge. Then, as each chromosome divides, the daughter chromosomes repel each other and the initial anaphasic movement is thereby given. But then arose a great difficulty. In completing a movement to the poles the chromosomes would perforce be moving against the repelling force of the centers, which originally had been powerful enough to force them into an equatorial position. There was thus no escape from the conclusion that this final movement presupposed a change in charge on the part of the center which altered it from a repelling to an attracting, or to at least a neutral, point.

Now it must not be forgotten that reasoning by analogy had of necessity played a large rôle in this hypothesis, so that the cautious cytologists of the day had never felt altogether safe in following Lillie. And thus, when in addition to this Lillie postulated a shifting or alteration of electric charge, most cytologists refused to follow. The matter was finally dropped by common consent as being altogether too involved and complex.

The abandonment of the dynamic hypothesis seemed to close the last avenue toward solution and for some fifteen years (roughly from 1912 to 1927) the whole problem lay virtually untouched. In the latter year Bělař reopened the question and his starting point was the pushing force involved in an expansion of the interzonal region between separating chromosomes. He followed this beginning with a series of brilliant investigations, but after some four years he reached a position that was surprisingly old-fashioned and new only in the sense that it brought together several opposing old hypotheses. In essence it

postulated a combination of the pushing force of Drüner, as given by the expansion of interzonals (Bělař's *Stemmkorper*), and the pulling force of Van Beneden, as given by the gliding and contraction of certain other elements in a poleward direction (Bělař's "*Zugfasern*"). But what forces were involved in the expansion of the *Stemmkorper* and what in the contraction and movement of the half spindle elements were questions that Bělař left for the future. However, his work had served as the stimulus to fresh courage and to a renewed attack on the problem.

Lamb's demonstration of the action of the oscillation of two isolated centers now received reconsideration, and certain other hypotheses were advanced. Thus may be mentioned that of Schaeede, which utilized the streaming of protoplasm as a moving force; the conclusions of Bleier and Metz accrediting autonomous powers to the chromosomes and involving accompanying action in the protoplasm surrounding them; Wassermann's idea of viscosity changes originating in the equatorial region and pushing daughter chromosomes toward opposite poles. Objections can be voiced to all these suggestions, and admittedly not any one of them is a finished product. But whatever their future fate may be, these various arguments and renewed investigations (and many seemingly irrelevant observations) have made us more certain of some of the dubious facts of 1905, have definitely eliminated others, and have finally brought forth some new ones.

One of these early observations now made secure is that the initial separation of daughter chromosomes is independent of the amphiaser. Whether the split chromosome is near the spindle or not, whether it shows spindle fibers or not, be it at right angles or parallel to the spindle axis, when the time for anaphasic movement arrives the daughter chromosomes separate and go in opposite directions. The process certainly is autonomous, and there is no simpler way to describe it than to say that the daughter chromosomes appear to repel each other.

Similarly, there seems no escape from the conclusion that whole chromosomes also repel each other, as evi-

denced not only by their position in the late prophase nucleus but also by their behavior toward each other on the metaphase plate.²

At the same time the cataphoresis experiments of Lillie, Dahlgren, Pentimalli and Churney and Klein seem to demonstrate that the chromatin of the nucleus is electro-negative during most phases of the mitotic cycle. I use the word "seem" advisedly, for in such experiments the danger of alterations in the affected cells due to injury from the electric current is very difficult to control. But taken as they stand, if these findings may be drawn together then at least the initial movements of chromosomes might well be ascribed to the charge carried by them.

Again, the old observations on the centers still seem valid. When the centriole divides, the daughter centrioles separate and travel to opposite sides of the nucleus, their paths being delimited only by the seeming necessity of remaining within a certain distance of the nuclear membrane. I may say here that it seems to me a minor issue whether a centriole be visible as a morphological entity or not. Certain it is that some centralizing factor is present even where no centriole can be demonstrated.

More recently we have discovered that every chromosome exhibits a specially differentiated region whose optical properties and staining reaction suggest a relationship with the centriole. This body of many names—let us call it the kinetochore—is without question deeply involved in the mitotic movements of the chromosome. Except in some very special and rare instances it is this part that precedes in the advance of the chromosome toward the pole. However, if the relationship that is so evidently suggested by this movement be one of attraction between centriole and kinetochore it should be pointed out that it is not always present throughout a mitotic cycle. Thus there is every indication that during a certain phase

² Though perhaps far-fetched it may not be amiss to point out that a mutual repulsion of bodies is open to at least one other explanation than that offered by electrical charges. Thus two bodies may repel each other if both are diffusing strongly—as in the case of two pieces of camphor floating on water.

of meiosis, the bouquet stage, it is not the kinetochore region that shows attraction toward the centriole but the free ends of the chromosome. It is therefore patent that either the kinetochore or the centriole is subject to certain changes that affect their relationship to each other.

I am drawing attention to these observations because they once more point to a dynamical hypothesis such as was proposed by Lillie in 1905. To be sure the difficulty encountered by Lillie is still present. If it is assumed that chromosomes and centers are negatively charged, the initial separation of daughter chromosomes may well be due to mutual repulsion. But the last part of the anaphasic movement still remains to be explained, for, as already indicated, it would take place against the force exerted by the negative center. So far as I can see, there are just two ways of escape from the difficulty: one, that the negative charge of either chromosome or center changes to a positive one or else becomes weak or neutral, while the movement is going on; the other, that, unlike the main mass of the chromosome, the kinetochore is positive and thus is not repelled by the center. But analysis will show that even in the latter contingency some degree of alteration in the charge would probably be called for. In short, the key seems to lie in the possibility of a change in the electric charge of one of the elements involved while the process is going on. I am not sure that this is altogether impossible.

No matter how we may twist and turn, however, we are faced with the realization that the whole process is very complex. Darlington, who has recently attempted an explanation on these lines, is forced to make so many assumptions that I do not feel very safe in following him. It must be remembered that every dynamic hypothesis has to reckon with factors that are given by the structure of the cell and these may be independent of any electrical action. Thus the expansion of the interzonal region seems an extremely important part of mitosis in at least some forms and I can not easily use it as a cog in any dynamical hypothesis. Again, certain forms point to a multiple

origin of the spindle, which is at present difficult to reconcile with the claim that the centers carry a charge. But such difficulties are self-evident, and I mention them merely to show that an explanation resting solely on electrical phenomena is going to encounter difficulty. Indeed the necessity for much further work is self-evident, for there are few problems that so urgently call for more well-established facts.

Finally, I should like to mention valid objections to any hypothesis involving the mechanism of a polarized or electrostatic field. These objections lie chiefly in the existence of lines of force in a system with three poles. Tripolar spindles certainly exist and the diagonal spindles of quadripolar figures also have a bearing. But in this connection it may not be amiss to point out that currents or lines of force such as are implied by an electrostatic field are not absolutely necessary to the hypothesis. Teorell has indicated that in an aqueous system where an electrolyte is diffusing steadily, an electrically charged particle like a chromosome would certainly be moved. If it is negative it would progress toward the opposite part of the diffusion potential field, and *vice versa*. Now in such a system no cataphoresis effect is involved and no current is flowing. This might remove some of the basic difficulties encountered by the electrostatic set-up that I have mentioned and yet not alter the rest of the reasoning.

I should like to repeat what I have said earlier: We must reconcile ourselves to the realization that the whole process is complicated. Even if electrical forces are mainly involved, there can be no question that modifications due to cellular conditions will have to be taken into account. Perhaps I am not altogether devoid of hope because I have become resigned to the thought that a solution will not be made by a single brilliant stroke. We all hope to make such a brilliant stroke in the course of our work and it is not without a certain sadness that one sees the possibility fade. But it may be that there is here at last an instance where a philosophy of resignation may lead us out of the wilderness.

THE PROBLEM OF CELL INDIVIDUALITY IN DEVELOPMENT

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BEFORE considering its embryological implications, let us first scrutinize the concept of cell individuality in the developed organism, from which it was originally derived. When Schwann says, "Each cell is within certain limits an individual," his qualification "within certain limits" seems to disavow the doctrinary rigidity which some of his followers have injected into the cell theory when they proclaimed that anything that ever happens in an organism is the resultant of individual cell activities. The severe and scornful criticism which these extreme "elementarians" had to take from the opposing camp of extreme "wholists" who wanted the attribute of individuality strictly reserved to the organism as a whole, is too well known to need further comment here. Much of this fight was carried out on philosophical grounds rather than on the factual grounds of observation and evidence. Many arguments advanced for or against the universality of the cell concept were merely rationalizations of beliefs of their authors that an organism ought or ought not to consist of discrete elements.

It is doubtful, for instance, whether the question of contiguity *versus* continuity among the elements of the nervous system would ever have become such a perpetual issue but for the fact that one group needed membranes and the other group "through" connections to explain nervous function as they saw it. Similar preconceptions were allowed to intrude into the problem of plasmodesms, that is, protoplasmic connections between cells. Those who think of protoplasmic communication and control as transportative in nature would rather have them, while transmissive theories can very well do without them. The observed facts themselves conspired with speculations on both sides by affording the expedient of artifacts caused

by histological treatment: If a preparation shows cell bridges, one can not always be sure that they might not be coagulated intercellular matter; and if a preparation shows no cell bridges, one can never be sure that they might not have been present in the living but ruptured by violent contraction during fixation.

As these few examples indicate, there has been a definite tendency to rate cellular individuality by the criterion of morphological discreteness. Insularity of cell behavior seemed to presuppose completely closed boundaries as of an island, and the evidence for this was sought in uninterrupted microscopic delineation. Obviously such an attitude is unwarranted in view of present-day biological knowledge which has stripped the microscopically visible of its former prerogative as vital standard and has pointed us more and more toward the ultramicroscopical and molecular realm. A submicroscopical boundary along which colloidal and other physico-chemical properties of the protoplasm change is no less real and physiologically effective, even though the microscope fails to reveal its existence. Nevertheless, some of the old practices are still with us, and to this day much of the discussion of cell individuality continues to revolve around microscopical arguments.

From this angle, however, the question is unsolvable. There is as much evidence for the existence of discrete, well-demarcated cell individuals as there is for that of plasmodia, large protoplasmic continua containing numerous nuclei but no visible cell limits to fence off nuclear domains. Free migratory cells are clearly unicellular individuals; but the heart muscle is clearly a syncytium; so is the blastoderm of the insect egg—to mention only a few prototypes. The occurrence of both protoplasmic continuity and protoplasmic fragmentation has been amply demonstrated. But more than that: it has been demonstrated that either condition can change into the other. Morphological delineation has thus turned out to be a rather inconstant character and by no means a true

test of cellular individuality. Cell bridges can break and reform; symplasms can divide up into cells; and cells can merge again into syncytia. Let us choose three examples to illustrate these facts, one from the lowest and the other two from the highest group of animals.

(1) In continuation of earlier studies by Wilson, Galts-off and others, Brondsted has recently made a thorough reinvestigation of the problem of cellular individuality in the reconstitution and germination of sponges. His observations leave no doubt that cell discreteness is of a transitory character, which comes and goes according to circumstances. Repeatedly, cells merge into large plasmodial masses, thereby losing their outlines, and later emerge again as individualized, well-circumscribed units. Free circulation of granules and other inclusions between the formerly isolated cell body and the ground substance into which it has opened, attests to the disappearance of morphological cell boundaries in the act of fusion.

(2) The syncytial character of the mesenchyme of vertebrates has been asserted by Rohde, Hueck, Studnička and others; again it has been recorded that under certain conditions nucleated parcels of protoplasm emancipate themselves from the syncytial continuum as mobile cells which can probably later become reincorporated in the common plasmodium (v. Möllendorff).

(3) Perhaps in no other tissue has the dogma of the morphological discreteness of the cellular individual been so vigorously defended as in the nervous system. As for the embryonic origin of the nerve fibers, the neurone doctrine has come out victorious, inasmuch as the neurite of each nerve fiber has been found definitely to be the product of a single discrete nerve cell. However, evidence of secondary protoplasmic anastomoses of the individual units keeps steadily accumulating. To the best of our histological knowledge, particularly according to Boeke, the terminal branches of a motor nerve fiber pass into the protoplasm of the muscle fiber without morphological interruption. Similarly, the connection between a cutaneous

nerve fiber and its sensory end organ has been described and depicted as intraprotoplasmatic. Anastomoses among nerve fibers were observed in the living object by Speidel and in tissue culture by Guiseppe Levi, who otherwise is one of the staunchest supporters of the individuality of the neurone. In nerve regeneration the newly outgrowing nerve fibers merge with the protoplasmic syncytium of the so-called cords of Büngner and only later become set off from the matrix and from one another by insulating sheaths.

Apparently, cell contour is a much more variable character than one would have anticipated. It can fade and reappear. But does this upset the concept of cell individuality? I do not think it does. In the studies on sponges quoted above, Brondsted has reported that even after merging into plasmodia, the different types of constituent cells can still be distinguished by differences of their nuclei and perikarya, and when a cell becomes released again, it behaves true to its original kind. Evidently, cellular individuality can survive protoplasmic confluence. We may adjust our picture of the cell to this situation by de-emphasizing the criterion of delineation. The only definitely discrete element in a cell is the nucleus, and since each nucleus keeps protoplasm within a certain radius under its control, protoplasmic territories have the value of cells, no matter whether their boundaries are marked by visible surfaces or merely by a change of physiological properties along the border. Any change in the colloidal consistency of the protoplasm attended by biochemical and bioelectrical differences will necessarily produce a definite orientation of ultramicros along the boundary and create some sort of physiological barrier and some degree of physiological isolation.

Within these limits, the cell is an individual, anatomically and physiologically speaking. But what about the cell in development? Let me briefly outline the crux of this problem also.

At the end of development we are confronted with a

unitary organized system, called an "organism," which, at the same time, is a collective of cells. At the beginning of development we find just one primordial cell—the egg. We call a system "organized" when its multiple elements appear in typical diversity, typical spatial distribution and typical temporal order. The elements are subordinated to this order and their freedom is restricted by it; hence, the order is a supra-elemental property of the system. In the developed system, "organism," the cells represent the elements; hence, organization is a supra-cellular property. But the primordium of the organism—the egg—does not consist of cells. Now, there arises a dilemma. Either the egg already possesses supra-cellular organization of the same order as the later body—then it is not just another cell, but an uncellulated organism; or it is merely a cell like others—then it can not be at the same level of organization as the later body. In this case, development would create organization of a higher order. It is to this latter view that the cell theory has committed itself. In the words of Schwann, "the individual cells so operate together in a manner unknown to us as to produce a harmonious whole," and the stress lies on "produce." Cells springing forth from repeated divisions would join hands, as it were, as equal participants in the building up of an organization all of their own making. The organism would be synthesized by progressive integration of cells into higher units, tissues, organs and the body as a whole. Cells would form the organism.

This view has met with vigorous opposition, culminating in a number of pronouncements about the inadequacy of the cell theory of development. In his address at the World's Columbian Exposition, Whitman argued the case in the most trenchant manner. One must realize the philosophical implications of the problem. If organization was to be accepted as something created *de novo* in every ontogeny, some principle had to be invoked which could mold order out of chaos, and the resort to vitalistic agents, such as Semon's "Mneme" and Driesch's "Entelechy,"

was a logical outcome. Faced with the alternative, the assumption of some primordial organization inherent to the egg seemed to many a much more palatable solution. Thus, the egg was vested with organizing powers representing the supra-cellular organization of the later organism, and research was directed toward the establishment of external signs of this organization. The egg and the young germ were considered as primarily integrated wholes within which parts gradually arise by individuation. At no time would the cells constitute independent units, but from the very beginning they would be subordinated to the actions of the organism as a whole. The cells would not form the organism, but the organism would break up into cells.

Clearly the two opposing views represented a modern edition applied to organization of the old antithesis: epigenesis *versus* preformation. Epigenesis of organization was the claim of the "egg-equals-cell" theory, while preformation of organization was the tenet of the "egg-equals-organism" doctrine. The latter soon gathered momentum from experimental evidence. Lillie showed that activated eggs of a worm, *Chaetopterus*, when prevented from cleaving into cells as in ordinary segmentation, still underwent a considerable degree of differentiation, involving development of parts within the protoplasmic continuum of an undivided egg. Localized differentiations of the egg cytoplasm of ctenophores, annelids, molluscs, insects and amphibians became known which imparted definite substantial and dynamic properties upon the cells to which they happened to become apportioned during cleavage. The cellulation of the egg was gradually recognized as a sort of epiphenomenon superimposed upon the differentiation of the germ rather than instrumental in its production. More and more one became impressed by the fact that the organization of the germ as a whole has stability as such, regardless of the extensive fluctuations to which its cells are subjected in nature and experiment. The individual cells began to appear as slaves, rather than

bosses, of the organism. The existence of individual cells as units was still acknowledged, but their rôle in embryonic organization was strongly de-emphasized.

As so often happens, however, in the wake of this sound reaction to exaggerated claims of the cell doctrine, an equally intransigent anti-cell doctrine raised its head. It tried to deny cellular individuality altogether and advanced a veritably totalitarian concept of development. Cells were ignored. The mass of the developing organism was considered as clay in the hands of the sculptor, passively submitted to molding forces which neither respect internal boundaries, nor admit of constitutional autonomy of individual units. If to the extremists of one side the individual cell was all and everything, to the advocates of the other extreme the organism as a whole appeared from the beginning in unchallenged control, cells or no cells.

Experimental embryology has, on the whole, steered clear of the two extremes. But it was difficult for the issue to find its proper level so long as one put the problem in terms of an alternative: Is the egg a cell or an organism? Is development epigenetic or preformed? Do the cells establish the properties of the developing organism or does the organism determine the properties of its cells? And so on. As we now see them, these questions are about as pertinent as if one asked: Has the face of the earth developed by volcanism or by erosion? The face of the earth is a highly complex affair, and so is its development. So also is the development of an organism. The time has passed when one could speak of development as if it were a single simple unitary phenomenon, like lightning or crystallization or the casting of a mold. Experimental analysis has revealed that what, in one word, we plainly call "development," is in reality an intricate combination of innumerable component processes, diverse and often disparate in character, which merely simulate oneness in that they all affect the identical material system—germ. Of course, it has been customary all along to single out growth (meaning increase in mass) or differentiation (in-

crease in diversity) or morphogenesis (elaboration of shape) and the like; but there has been a general feeling that all these features are manifestations of a common principle, and that to separate them was pardonable only as an act of mental abstraction. The truth, however, is that they are essentially separate phenomena, and, in fact, each one in itself highly composite. Nuclear division, cell growth, cell division, cell aggregation, movements of cell complexes, differential growth, cytological differentiation, polarity, orientation—these are only a modest selection from the list of component phenomena into which we have learned to decompose development.

The revelation of the multiplicity of developmental processes and mechanisms has been a sad disappointment; for it has removed all hope of a general, comprehensive and universal formula of development. At the same time, it compels us to ask every question which formerly was aimed at development in general, separately for each one of its manifold components. We no longer ask: "Is development epigenetic or preformed?", but focus on a single contributory phase, asking: "How much of it is due to epigenetic and preformed conditions?", only to find that the answer varies with the object. It is this abandonment of the unitarian claim which has rendered us immune to both the strictly elementarian and the strictly totalitarian view, and which has steadied our picture of the relative rôle of cell and organism in development. Instead of a sweeping generalization, we expect a precise description of just how much of a given developmental phenomenon is due to active participation of the cells and how much to effects of supra-cellular order; what does a cell do, and what is being done to it, in a given phase of development? These are questions with which one can deal in matter-of-fact fashion, without even touching the sore spots of principle.

A few specific cases may serve as examples. Let us consider, first, histological differentiation. Do cells produce specific histological characters by intrinsic capacity

or through external influences? For instance, is the elaborate conducting and contractile apparatus of a cross-striated muscle fiber developed by virtue of a constitutional property of the myoblast cell or can muscular development perhaps be imposed upon any protoplasmic mass by proper influences from its surroundings, as has been claimed by Carey? Observation and experiment have answered in no uncertain terms: Cellular differentiation is founded on innate properties of the cells themselves.

To prove the point, we remove cells from the community of the organism, thus depriving them of possible outside directives. We choose cell groups of an early germ with no manifest signs of differentiation, explant them into an extraneous medium and watch their fate. Morphogenetic development remains poor. But histologically, the explanted cells differentiate with amazing perfection. As Holtfreter and others have shown, they give rise to typical nerve cells, pigment cells, muscle cells, cartilage cells, notochordal cells, goblet cells, pronephric cells, etc. These productions are absolutely definite and discrete, each cell differentiates distinctly into one type or another, and there are no intergradations, hybrids or blends between the established cell types. Obviously, even very young cells "know" how to make a muscle fiber, a neurone, a chromatophore, etc., and we may conclude that the mechanisms for histological differentiation belong to the pre-formed endowment of a cell.

The same experiments have revealed, however, that cells are by no means single-tracked from the beginning. We know approximately what is to become of any given cell group of an early germ during normal development. Now, one has often noted that cells, when reared in isolation, can deviate considerably from their normal fate. Presumptive nerve cells, for instance, can become muscle cells or chorda cells, and the like. This means, evidently, that each cell of the early germ possesses a definite repertoire consisting of several discrete differentiation po-

tencies. A limited number of clearly circumscribed courses are open to each cell.

In isolation, chance may decide which course is actually followed. But inside the organism, the choice is definite: there, each cell develops in conformity with the character of its surroundings. It becomes a cone or rod when in the retina, a cartilage cell when in the center of a limb bud, and a neurone when in the brain. Intrinsically capable of a variety of performances, the cell receives some definite cue from the locality indicating which trend it is to follow. These cues are decidedly of supracellular origin. Their effects have been beautifully demonstrated by transplantation experiments for which the schools of Spemann and Harrison have become famous. Transplant a young and undifferentiated cell group into the region of the head, and it will form eye or brain; transplant it to the anterior trunk and it will form limb, or further back, kidney; and, transplant it to the rear, and it will form tail—the same cells forming different structures depending on their locations. We may say: “Organizing factors take hold of the cells and direct them to appropriate formations.”

We must be careful not to lapse again into the erroneous metaphor of the sculptor molding clay; let us stress, therefore, that no organizing factor has yet been observed that would have made cells assume histological structures strange to their inherited repertoire. This statement is based on crucial evidence obtained from transplantations between different species and orders of animals. In provoking specific histological characters, organizing influences are bound, therefore, to operate through the cells as their executives, and the specific character of the execution is determined by the properties of the reacting cells. To this extent, differentiation is active cell work. But this is not the whole story: The factors which turn a given cell into a definite histological trend do not, at the same time, fix all the particulars of its future course. Take a nerve fiber, for example. The factor which turns an indifferent epithelial cell into a nerve cell does not, at the same

time, decide the spot at which the nerve fiber will leave the cell body; and the factor which opens the door for the fiber has no control over the further journey of the outgrowing sprout; and, again, the factors which map this course are different from those which decide where it will terminate. To put it drastically, the nerve fiber is elaborated in assembly plant fashion. In some phases of this sequence the individual nerve fiber plays an active role; in others it behaves purely passively. The original outgrowth of the sprout is free, guided presumably by oriented traffic routes of the surrounding body. But once the free tip has become hitched to a peripheral migratory cell, it is taken in tow and dragged to a destination no longer of its own choosing. It is not at all easy to tell how much of the winding course of a nerve fiber is due to active orientation and how much to passive distortion.

The same holds for cell shape in general. Part of it can be ascribed to autonomous transformations of the individual cell body, the rest to passive deformation caused by pressure, growth and spatial limitation on the part of the cell collective. With cell movements it is the same story: Free cells may aggregate in response to a local stimulus and thus form a crowd whose further growth and movement, as a whole, sweep the participant elements along without leaving them much further individual choice. Similarly, erstwhile free cells which secrete a cementing substance thereby imprison themselves and become subjected to all the dislocations of their common matrix. An opening or canal may be formed either by the active recession of the cells lining the prospective lumen or by the passive destruction of cells with subsequent resorption. When one sees pigment cells arranged in regular geometric tracts, one suspects that they have been forced into this alignment by the topography of their surroundings. But how much the pattern is really of their own making has been shown by Twitty when he interchanged the source of the pigment-forming cells between two species of distinctly different color patterns: the transplanted cells

assumed the distribution characteristic of the species of their origin rather than that of the host body. I would venture to say that what the individual cells actually bring into the deal in this instance is a tendency either toward dispersion or toward aggregation; whereas the loci of aggregation in the latter case are presumably a matter not of the cells themselves, but of their matrix, so that the resulting pattern would, again, be of composite origin.

I have deliberately dwelt on these varied examples, in order to make clear that practically every step in development reveals the cell in a double light: partly as an active worker and partly as a passive subordinate to powers which lie entirely outside of its own competence and control, *i.e.*, supra-cellular powers. Now, it is perfectly true that some of these latter result from interactions of cell individuals and are, therefore, of cellular origin. But it is equally true—and the findings of experimental embryology are one rich store of evidence for our assertion—that many of them are supra-cellular from the beginning. They are those organizing conditions through which the fate of the individual cells—undecided, as we have seen, at first—is guided, controlled and progressively fixed. They are definite at a time when the individual cell fate is still indefinite. They impose order upon what otherwise would be an anarchic cell chaos. They are inherent properties of the living system, germ, as a whole, in contradistinction to the inherent properties of its constituent cells of which we have spoken before.

One frequently refers to these organizing entities under the term of “fields.” Their existence can be traced back to the egg. In fact, just as there is a continuity of cells from the egg to the organism through successive cell divisions, so there is continuity between the primordial organizing fields present in the undivided egg and the localized fields of the later germ. Primordial fields segregate progressively into more restricted fields, and, furthermore, induce new fields in neighboring areas of the germ. Thus,

the organizing principles of a germ have an ontogenetic history of their own which is not cell history. Their possession marks the egg as an entity of the rank of the organism; this, in answer to a question put above. Their development is a matter of the developing system as a continuum, like tensions, currents, potentials, and the like, and they pay no heed to cell boundaries, although sooner or later the intricate interplay between them and the cells sets in, of which we have spoken before.

The existence of these primordial organizing principles in the egg has been firmly established by modern experimental embryology. No pure cell theory derived from the developed organism can embrace them, unless by a vicious circle.

In conclusion, we may say that the cell theory is correct: The egg is a cell and it gives rise to all the successive cell generations which contribute to the organism. But the organismic theory is likewise correct: The egg is also an organism, and it passes its organization on continuously to the germ and the body into which it gradually transforms. Only this dual concept seems to fit the facts, as we see them at present. To be consistent, we should supplement Virchow's well-known tenet of the cell theory: "*Omnis cellula e cellula*," by its counterpart: "*Omnis organisatio ex organisatione*." If the former denies spontaneous generation of living matter, the latter denies spontaneous generation of organization. In admitting this, we merely paraphrase what Whitman has called the "continuity of organization." But within these specified limits the cell, even in development, is still, as Schwann has said, an individual.

WHAT OF THE FUTURE?

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SPEAKING as a philosopher, and in search of the qualities which distinguish living things from the non-living, Bergson points out that the incorporation of time into the living system is its distinguishing characteristic. He expresses the thought in these words:

Like the universe as a whole, like each conscious being taken separately, the organism that lives is a thing that endures. Its past, in its entirety, is prolonged into its present, and abides there, actual and acting.

Wherever anything lives, there is, open somewhere, a register in which time is being inscribed.

If time is thus inscribed, where is the record and how is it preserved? That question has been largely answered by studies on the structure and behavior of cells. Obviously, organisms are brief and fleeting in existence—they can not represent a continuous record. But within them are the germ cells which do constitute an unbroken and constant series. Since, however, male and female germ cells differ widely in many respects, there must be comparable structures in the two which would represent this temporal record. It was not long until such a situation was demonstrated in the nuclear structures, now for so many years the objects of intensive study—the chromosomes. They were observed to reproduce themselves down to their ultimate visible parts with the utmost accuracy. The results of this appeared in the constancy of number, size, structure and behavior of chromosomes, even in large taxonomic groups. Here there is certainly some sort of a record persisting, obviously, over a period of time estimated at millions of years. But is it the precise inscription which will delineate the characters which we associate with organisms of specific types? Genetics has convincingly demonstrated that the record is complete, specific, accurate and persistent. In the final analysis, however, there are lacking the visible ultimate units, within the chromosomes, which genetical analysis calls for.

Confronted here with the same difficulty which physical scientists meet in their analyses, the biologists solve it in the same manner by the creation of supposititious units which they call genes.

A survey of the history of the cell doctrine shows accordingly that it demonstrated: (1) The universal presence of cells in organisms; (2) the differentiation of these into two types—somatic and germ; (3) a method of exact reproduction; (4) the presence within the cell nucleus of a continuous racial material; (5) an exact parallelism between the behavior of this material in the germ cells and that of somatic characters genetically; (6) the precise organization of the racial material in a linear order within the chromosomes; and (7) an exact relation between this chromosome organization and groups of somatic characters.

A continuation of these most fruitful coordinated lines of study will undoubtedly bring notable additions to our knowledge of cellular phenomena. At the same time the extension of the analysis of vital processes to invisible causal units introduces necessarily new methods of study. Here we find ourselves on somewhat the same ground as that occupied by physical scientists. Like them we must reach some conception of the nature of these imagined units. First we have to determine whether our search lies directly with the physical scientists or whether, accepting all the aid they can give us, we finally depart into fields of our own where new guides and methods are required. Their own experience seems to point the way.

The physical scientists have discovered a series of at least three systems of increasing complexity, each having characteristic units whose properties explain the operation of the system. The units of the most complex order are constituted of those of the next, and these, in turn, of the simplest. Molecules, atoms and corpuscles are thus related. Three important things are here to be noted: (1) The genetical continuity of the series. (2) The specific relation existing between the character of a unit and

the phenomena of its system. Chemical activities are explained directly in atomic terms, but atoms can not here be dissociated from corpuseles on one hand or from molecules on the other. At each level in the organization of matter there are apparent characteristic phenomena, and to explain them there are conceived appropriate units. (3) The state of matter, or the nature of the successive combinations of units to form more complex ones, depends upon the conditions associated with the system, notably temperature. On the earth, where a relatively limited temperature obtains, atomic and molecular organization prevails.

When we come to living systems, such as cells, we encounter new phenomena, slightly or not at all represented at lower levels. To explain these, two antagonistic theories have been held. On the one hand it is assumed that vital phenomena are so peculiar that entirely new forces must be conceived to explain them; on the other hand it is postulated that they are not unique, but that the laws of physics and chemistry alone are entirely sufficient to account for them. Since living things have in them only such chemical elements as are found in the non-living, it is impossible to conceive of them as entirely unique and apart from the rest of nature. Likewise, because they manifest phenomena all their own, it is hardly possible that they should have the same organization as the non-living. If unique phenomena require novelty of organization and if the nature of an organization is dependent upon its constituent units, then it must be concluded that there are characteristic living units. Further, if these are not merely molecular, as the argument indicates, then they must be produced by an appropriate combination of molecules. Further, vital phenomena are possible only under limited and unusual conditions, realized possibly on this one planet alone.

Physical scientists in search of an understanding of their units note the constancy and limitations of the phenomena to be explained. Everywhere in the universe

appears the same limited series of chemical elements with the same precise seriation. The symbol of constancy in relations, a circular orbit, typifies the path of one unit or component about a fixed center or another sort; additions of components, increasing the complexity and weight of the whole, are sufficient to provide the series of chemical elements. Combinations of these relatively simple atoms in accord with their inner nature suffices to produce the much greater range of molecules which characterize compounds.

When the biologist turns to a survey of the properties of the system with which he works, he discovers very different conditions. Instead of constancy there is almost limitless variability in the aggregates which he studies. Where the chemist views comprehensible limitations to his elements and compounds, the biologist is confronted by a procession of almost infinite steps of gradation from simple to complex. In the inorganic world there are but slight possibilities of succession in time; in living things it is their very substance. Non-living things are relatively independent of their surroundings; living things depend for their very existence upon close and constant interaction with their environment. Finally, living systems have unique properties not elsewhere represented—metabolism, reproduction, coordination and consciousness. If, as assumed, units are so constituted as by their nature and action to explain the phenomena of systems. is it possible that atoms and molecules, as such, can account for vital phenomena? It seems impossible that this can be true. Bergson clearly states this contrary position in the following words:

“We do not question the fundamental identity of inert matter and organized matter. The only question is whether the natural systems which we call living beings must be assimilated to the artificial systems that science cuts out within inert matter, or whether they must not rather be compared to the natural system which is the whole of the universe. . . . The real whole might well be, we conceive, an indivisible continuity. The systems we cut out within it would, properly speaking, not then be parts at all; they would be partial views of the whole.

So of life and of the physico-chemical phenomena to which you endeavor to reduce it. Analysis will undoubtedly resolve the process of organic crea-

tion into an ever-growing number of physico-chemical phenomena, and chemists and physicists will have to do, of course, with nothing but these. But it does not follow that chemistry and physics will ever give us the key to life.

How can the origin of living units be conceived? There is but little positive knowledge to which we can turn for an answer to this question, but there are some suggestive facts which may point a way toward better knowledge. Since to preserve their existence such units must constantly react with their surroundings, it is not unreasonable to suppose that they owe their beginnings to such a circumstance. But if they did not exist, how could they react? Their very nature is one of becoming something different. They are made up of a few chemical elements and they depend for their existence and, therefore, presumably for their origin, upon a few definite conditions of radiation, temperature, moisture and chemical compounds. Under the impulse of these peculiar conditions it may be supposed that approaches to a specifically reacting unit were made and are still being made. Change is the essence of a reaction system and must have been determinative in its origins.

A single unit, conceivably, would be sufficient to establish a living system, for it would necessarily have the properties of the whole—assimilation, reproduction and coordination. The change in character is here always unidirectional—from simple to complex—the addition of more parts with increased diversity of reactions. When genes are studied they show a linear organization into aggregates. If the beginning of a system was a single gene we may assume that by transverse division it produced two, one proximal, the other distal, producing thus a polarity in relation to the environment. A continuation of this process, which obviously was infrequent and probably reactive, would result in a chain of genes. Such gene strings are identified with the linearly organized chromosomes. Inherent in any such an arrangement is succession or time. Some genes are younger than others, and it

may be supposed that their specific operation in the system would correspond in time.

Gene strings, as we know them, are parts of cells, and it is a far cry from a few genes to a complete cell. Far from being a simple thing a cell is very highly organized. It must have required a large proportion of organic history to evolve such a structure. Ordinarily when we seek a guide to succession in organic changes we try to find it in the sequence manifest in a phylogenetic series. Unfortunately, there is little to indicate the steps in cell development. Probably there are more than we know about, but almost certainly they will be found among low plant forms. It would not be difficult to conceive the formation of a nucleus from a chromosome, for this can be seen in many mitoses, but the origin of a cytosome does not seem to be so readily suggested.

When a system is inaugurated and operative it is not so difficult to understand its further development and increased perfection, but it is quite otherwise when the origin of the system is involved. A cell in general is a comprehensible structure. Its parts are differentiated and their peculiar functions are somewhat indicated by their relative positions and constitution. But how did they come into being and what is the nature of their integration? Why a nucleus and a cytosome? The relation between chromosomes (or genes) and body characters can be analyzed and predictions made regarding new manifestations, but how is the course of events between the single-celled zygote and the innumerable-celled adult determined?

The chief fault in our thinking with regard to the nature and function of determinative units in development comes from an over-simplification of the problem. From the beginning there has been a strong tendency to conceive a determiner as some factor of single and unique power operating only at some particular time. Weissmann carried this to its logical conclusion and imagined his determiners sorted out during development until finally only one kind remained in the completely differentiated cell.

As we now clearly perceive, the actual condition is just the opposite of this. There is no process of sorting out; characters do not result from the operation of single controls, but depend upon the effects of all of them; one determiner has many effects; these effects differ according to the time in the series at which factors operate; each change in the system is directly dependent upon those which have preceded it; time is thus an integral element of the system.

Our studies have led us from an understanding of the general nature of cells to an analysis of the structure and function of their parts. The progress so far has been steady and consistent, and it does not seem probable that any extensive revisions of factual statements will be called for. Theoretical interpretations likewise, in general, seem soundly based. What the future calls for is mainly an extension of the lines of investigation so far pursued, with much more emphasis upon a synthetic treatment of accumulated facts. In particular the coordinated activities of cell parts must be systematically studied so that a comprehension of the functioning whole can be reached. The method required here is obviously not that of avoidance, through appeals to an incomprehensible organismal concept, but a refinement and extension of the analytical treatment which has led us to our present position.

From the fact that analysis has finally brought us to the point where we can no longer see the units which seem required to explain cell functions, we are faced with the necessity of cultivating more strongly our powers of interpretation. Above all, we must comprehend the unique nature of these determinative units. If non-living units are relatively fixed, limited and precise, how are living units which present opposite characteristics to be conceived? What, in their nature, would lead to constant variability which, in general, is unidirectional in character? How are they organized to preserve a record of their experiences with elements external to themselves? Answers to such questions as these, I believe, will indicate the direction in which the cell doctrine will be extended in the near future.

THE SIGNIFICANCE OF POLYPLOIDY IN PLANT EVOLUTION¹

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OF the numerous processes which have been active in plant evolution, only one is well enough understood from the cytogenetic point of view so that we can give a safe estimate of its rôle in species formation. That is the process of chromosome doubling or polyploidy. The effects of this process have been so far-reaching, and it is so wide-spread, that any one who would have a real understanding of species interrelationships and species evolution in the majority of plants must realize the significance and implications of polyploidy and the processes that accompany it.

To understand this phenomenon, we must first of all know what are the effects of chromosome doubling without any other change in the genetic make-up of the plant. We can do this by comparing the morphological and physiological characteristics of a diploid plant with those of a polyploid that has been produced from it artificially, by doubling the somatic chromosome complement. Unfortunately, our understanding of these artificially induced polyploids is one of the weaker links in our chain of evidence concerning the nature of polyploidy. This link, however, is now being very rapidly strengthened, thanks to the efforts of Blakeslee (1937), Nebel and Ruttle (1938), Greenleaf (1939) and others, who have given us easy ways of producing polyploids by means of colchicine and indole acetic acid. The most important facts obtained from a study of artificially induced polyploids may be summarized as follows:

1. The size of the cells is increased.
2. The water content of the cells is increased, as was

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shown first by Wettstein and his students (Becker, 1937) in mosses, Schlösser (1936) in a wild strain of tomato, and by Hesse (1938) in *Petunia*. This higher water content is associated with a lower osmotic value of the cell sap, and makes the tetraploid less resistant to frost than the diploid.

3. The evidence on growth rates is conflicting. Many workers have reported slower growth in tetraploids, but the only two quantitative experiments that have been performed, those of Fabergé (1936) on the tomato and Hesse (1938) on *Petunia*, failed to show significant differences.

4. The size of some of the organs is usually increased. This increase is most evident when diploids are compared with tetraploids. Higher polyploids do not usually show any further increase in size and may even be dwarfed.

5. The organs become shorter and broader. This is true of the leaves of *Solanum*, *Lycopersicum*, *Datura*, *Nicotiana* and other plants, and of the fruits of *Datura* and the Cucurbitaceae.

6. Hesse (1938) found that in *Petunia* the autotetraploid was less branched than the diploid.

7. The time of blooming is delayed (Hesse, 1938) and the period of blooming appears to be prolonged.

This information on the immediate effects of chromosome doubling is as yet based on relatively few plants, most of which belong to a single plant family—the Solanaceae.

In addition to these morphological and physiological effects, we know of two other very important results of polyploidy. In the case of recessive or imperfectly dominant mutations, the visible mutation rate in polyploids is very much reduced, since, as Haldane (1930) has shown statistically, the chances of getting a tetraploid homozygous for a set of four allelomorphs are much less than for the corresponding pairs in a diploid, for a hexaploid they are still less and for an octoploid practically negligible. Stadler (1929) has shown that the production of

visible mutations by means of x-rays is greatest in diploid wheats, less in tetraploid forms and least in the hexaploid.

Finally, the fertility of a plant is enormously affected by polyploidy. If the original plant is a fertile species, the polyploid derivative will be partially sterile, due to the formation of multivalent associations of chromosomes and their occasional irregular segregation. If, on the other hand, the diploid plant is a sterile hybrid, the polyploid produced from it is generally fully fertile. This last fact is the basis of the principle of allopolyploidy or amphidiploidy, the importance of which in the development of plant species can not be stressed too strongly. Equally important is the well-known fact that tetraploids are not only difficult or impossible to cross with their diploid progenitors, but also produce practically sterile triploid F_1 hybrids when crossed with them.

This brings us to the all-important fact, now so well known that I need not cite the numerous examples of it, that by means of chromosome doubling or polyploidy a sterile hybrid can produce directly a fertile, constant species. On this fact is based the chief classification of polyploids into autopolyploid and allopolyploid types. The definition of these terms, and the identification of the types which they represent has been subject to widely differing opinions among cytogeneticists. I believe that for practical purposes the best definition of an autopolyploid is a polyploid of which the corresponding diploid is a fertile species, while an allopolyploid is a polyploid containing the doubled genome of a more or less sterile hybrid. These are essentially the same definitions as those given by Lilienfeld (1936), but differ from those of Müntzing (1936, p. 311) and Darlington (1937, p. 183) in that less emphasis is placed on structural differences between and the types of pairing of the chromosomes. The criterion most often used for distinguishing between auto- and allopolyploids has been whether or not the polyploid forms multivalent associations of chromosomes at meiosis. This can, however, be very misleading, since we now know many more or less

sterile diploid interspecific hybrids in which the chromosomes pair almost or quite normally (*Primula kewensis*, *Tragopogon pratensis* \times *porrifolius*, *Paeonia albiflora* \times *temifolia*, *Crepis rubra* \times *foetida*, *Tradescantia*). These might be expected to produce allopolyploid derivatives which would form some multivalents. One of these allopolyploids, *Crepis rubra* \times *foetida*, may actually form a complete set of five quadrivalents (Poole, 1931). On the other hand, some autopolyploids, such as the tomato, may form very few quadrivalents (Humphrey, 1934; Upcott, 1935).

The difficulty with the definitions given above is that they are very difficult to apply. We can safely call a plant an autopolyploid if we know its origin from a fertile species, and are probably justified in calling it one if it falls within the range of variation of a diploid population that is interfertile or if it differs from such a population only in those characteristics known to be affected directly by chromosome doubling. An allopolyploid, likewise, can be identified if its parentage is known or if the identity of the parent species can be inferred on morphological grounds. Between these recognizable types, however, there is a long series of intermediate polyploid types, while still other polyploids are of completely unknown origin. Many of these can not be safely classified as either auto- or allopolyploid. In spite of this difficulty of definition, however, we must always keep in mind the fact that some polyploids are of hybrid origin, while others, often closely related to them, are not.

Bearing in mind these facts, we can now consider the effect of polyploidy and hybridization on a group of species. The simplest case, that of a single species and its autopolyploid derivative, is illustrated by the watercress, *Nasturtium officinale* (Manton, 1935). In this case the polyploid is morphologically within the range of variation of the diploid, except for some size differences. There are physiological differences, such as the slower rate of development and later time of blooming which have been found

in some artificially produced autopolyploids, and these give the polyploid race a different geographical range from that of the diploid. The two races have always been placed within the same species by systematists, and the question of whether they are to be considered as belonging to the same or to different species depends simply on how we define our species concept. The tetraploid is, of course, genetically isolated from its parent diploid, and might therefore be expected to pursue an independent course of evolution. This course is, however, unlikely. As I have mentioned, there is both theoretical and experimental evidence to indicate that mutation, as well as any other known evolutionary process, such as structural changes in the chromosomes, produces its effect on autopolyploids very slowly as compared to diploids. Furthermore, due to the fact that polyploid species are more infertile than their diploid prototypes, an autopolyploid is unlikely to maintain its purity unless it is completely isolated not only from its diploid progenitor, but from its polyploid relatives as well. Would such a completely isolated race, which would have to become highly inbred, and in which the visible mutation rate is greatly reduced, be likely to give rise to a new line of evolution? I doubt it.

The next case is that of a simple allopolyploid between widely different species, neither of which has in nature given rise to autopolyploid races. Examples are the classical cases of *Primula kewensis*, *Galeopsis Tetrahit* and *Nicotiana Tabacum*. In these cases, a new species has arisen at once. This species is completely distinct from either of its progenitors, and though it for the most part combines the characteristics of the latter, the new allopolyploid may occasionally possess some distinctive characteristics of its own. Since such allopolyploids contain fewer duplicated genes than autopolyploids, their visible mutation rate should be more rapid, although Stadler's (1929) experiments on *Triticum* indicate that it is nevertheless slower than that of diploids. Since, furthermore, allopolyploids usually have different geographical

ranges from those of their diploid parents, they are subjected to a different selective activity of the environment, and might therefore be expected to give rise to new morphological types through the processes of mutation and natural selection. The frequency with which this has taken place can be estimated from the proportion of genera and larger plant groups for which an allopolyploid origin can be inferred, either because their basic chromosome numbers are multiples or sums of lower numbers existing in related groups or because their basic numbers are so high that polyploidy is the best a priori explanation for their origin. The number of these genera is not large; they form about 16 per cent. of the genera of Angiosperm which are well enough known cytologically so that any inferences can be made concerning them. Most of these genera, moreover, are in complex families like the Rosaceae and the Malvaceae, and have close relatives with lower chromosome numbers. Probably the largest and most diverse single group of plants of which the allopolyploid origin is clearly established is the subfamily Pomoideae of the Rosaceae, containing the apples, pears, loquats, *Sorbus*, *Crataegus*, *Cotoneaster* and several other familiar genera. The basic haploid number nineteen, found in the poplars, willows, magnolias and grapes, the number twenty-three found in *Fraxinus*, the ash, and forty, the basic haploid number of *Tilia*, the basswood, are very likely of polyploid origin, but such cases are not the rule in the higher plants. Allopolyploidy appears to have given rise sporadically to new genera and perhaps even families, but does not seem to have been a significant factor in the production of the major plant groups.

The most complex, but probably also the most common situation in plants is that in which both auto- and allopolyploid types exist together in the same complex of related species. In this case hybridization between auto- and allopolyploid forms is almost certain to take place, and many of these secondary hybrid derivatives will be fertile enough to be self-perpetuating. If three, four or more

diploid species are able to form allopolyploids with each other, as is generally the case, additional allopolyploid types containing genes from three, four or more different diploids will be formed. Thus there arises a complex network of interrelated forms, which defies classification according to the usual concepts of the species. This has been designated by Professor Babcock and myself (1938) a polyploid complex. Such a complex consists of two or more diploids, which are well isolated from each other genetically, which therefore are, when taken by themselves, quite distinct from each other. Among the polyploids there may be autopolyploids which are nearly or quite impossible to distinguish from one or other of the diploids, except by counting their chromosomes; there are allopolyploids that are exactly intermediate between two diploids, and there are all sorts of secondarily derived polyploids. Thus among the polyploids the gaps between species no longer exist or are at least very much smaller and harder to recognize. Polyploidy, therefore, tends to break down genetic barriers and to permit exchanges of genes between genetic systems that in the diploid condition are completely isolated from each other.

As an example of a polyploid complex we may use the American species of the genus *Crepis*. In this group the complexity is increased by the presence of apomixis. This causes the polyploid portion of the complex to be split up into innumerable microspecies and permits the preservation of many "unbalanced" polyploid types—triploids and pentaploids—that in a sexually reproducing complex would be sterile and unable to perpetuate themselves. Otherwise, the interrelationships between the various types in an agamic polyploid complex, like that of *Crepis*, are essentially the same as those in a sexual polyploid complex. The *Crepis* complex has been described in detail elsewhere (Babcock and Stebbins, 1938). Other polyploid complexes are the genera *Zauschneria*, *Rosa*, *Rubus*, many sections of *Potentilla*, *Antennaria*, *Taraxacum* and scores of others. All of them are "critical" genera to the systematist. The

difficulty of these genera is intrinsic. The systematist need have no inferiority complex about his failure to find clear differences between species in them. Their complexity is the result of the preservation of scores or hundreds of allopolyploid and part allopolyploid types which are of hybrid origin, and which completely obliterate the gaps between what were once distinct or even remotely related species.

When we turn to the geographic distribution of a polyploid complex, we find this striking fact. In every case that has been studied, the majority of the diploid species are relatively restricted in distribution, while most of the widespread types are polyploid. In *Crepis* only one of the seven diploids has a wide range; the rest are confined to two small areas near the western edge of the range of the group as a whole. Furthermore, the strictly autopolyploid forms are almost as restricted in distribution as the diploids, while the farther edges of the range of the group are occupied by allopolyploid forms of complex origin. There is considerable evidence to indicate that these allopolyploids have been able to occupy a wide range because they have acquired a favorable new combination of the different physiological characteristics of their diploid ancestors. A good case of this is the *Crepis* population of the Mt. Hamilton range, in west central California. The climate of this part of the coast ranges differs from that of any of the regions occupied by diploid species of *Crepis* by possessing the combination of a mild winter and a hot dry summer. The two *Crepis* races found there are both allopolyploids, which, judging from morphological comparison, are derived from hybrids between one diploid that prefers a mild but relatively moist climate and one that grows in a cold, dry one. The Mt. Hamilton allopolyploids appear to have acquired tolerance of a mild winter from one parent and of dryness from the other. In other words, natural hybridization and natural selection have produced new types which grow under different surroundings from the old ones, just as the plant breeder produces new, better growing types by means of hybridization and artificial selection.

The relative importance of chromosome doubling alone as compared with recombination through hybridization as a means of increasing the range of the polyploid complex probably varies in different genera. In *Crepis* allopolyploidy is the important factor, while in *Tradescantia* (Anderson and Sax, 1936) the increase in distribution is the result primarily of chromosome doubling or autopolyploidy.

The center of distribution of the diploid species of a polyploid complex is naturally the center of variation of the complex as a whole. In the regions where two or more diploids grow together, new elements may constantly be added to the complex. In the polyploid complex of *Crepis*, for instance, the species are particularly variable and difficult to classify in northern California and adjacent Oregon; farther east and south the variants become fewer and fewer. The position of this center with reference to the complex as a whole varies in different genera; the relative distribution of diploids and polyploids is not always the result of the same climatic and ecological agents. In *Tradescantia* the diploids are chiefly southern and the polyploids northern, while in *Vaccinium uliginosum* (Hagerup, 1933) and *Campanula rotundifolia* (Böcher, 1936) the diploids are arctic and the polyploids more southern. In the Japanese species of *Chrysanthemum* (Shimotomai, 1933) the polyploids are maritime and the diploids inland types, while in the English forms of *Erodium cicutarium* (Warburg, 1938) the reverse is the case. In *Crepis*, the different diploids occupy widely different extremes of ecological habitats, and their center of distribution in northern California is a region in which several such habitats are found side by side. One important generalization which undoubtedly holds good for the bulk of polyploid complexes, including *Crepis* and *Tradescantia*, is that the polyploids are dominant in regions which have been only recently opened to occupation by plants, or which have been subjected to great climatic or other environmental changes, while the diploids tend to occupy the older, more

stable habitats. This makes the study of polyploid complexes very important from the standpoint of plant geography.

The distribution of polyploidy and polyploid complexes through the plant kingdom is strikingly irregular, and, except for its great frequency in certain families, shows no correlation with any phylogenetic scheme (Stebbins, 1938). Polyploidy is particularly frequent in the Gramineae, Polygonaceae, Nymphaeaceae, Rosaceae and Malvaceae, and in certain genera or tribes of the Ranunculaceae, Cruciferae, Labiateae and several other families. On the other hand, there are a few families, such as the Fagaceae, and several large genera (*Ribes*, *Ficus*, *Berberis*, *Philadelphus*, *Medicago*, *Lathyrus*) in which polyploid series are rare or unknown. In another group of families of monocotyledons, chiefly the Cyperaceae, Juncaceae and Iridaceae, the presence of polyploidy is obscured by the large number of aneuploid or non-multiple series found in them. On the other hand, there is a definite correlation between polyploidy and the habit of the plant (Stebbins, 1938²). Polyploidy is most frequent in herbaceous perennials and relatively infrequent in woody plants and in annuals.

Finally, we must ask what is the evolutionary history of polyploid complexes and what relation does this bear to the broader aspects of the evolution of genera and families of plants? In *Crepis* the answer is quite clear. Although there is much evidence to show that this complex has existed since the late Tertiary epoch, its polyploid members have not in all this time evolved any new characteristics. Although each diploid has some characteristics not pos-

² In this article the values for χ^2 were unfortunately calculated by the wrong method. The correct values obtained by the method recommended by Fisher, which included the combining of classes as given in Table I where these were less than 10, are as follows: Page 192, lines 44-46: $\chi^2 = 1.98$, $n = 2$, $P = 0.3-0.5$. Lines 49-50: $\chi^2 = 4.61$, $P = 0.05-0.1$. Last two lines of P. 192: $\chi^2 = 12.21$ and 13.54 , $n = 2$ (columns for 0-25 per cent. and 25-50 per cent. combined, also those for 50-75 per cent. and 75 per cent. +), $P = 0.01$. P. 193, line 5, $\chi^2 = 8.1$, $n = 1$ (columns combined as in last), $P = 0.01$. Lines 7-8: $\chi^2 = 4.2$, $n = 1$, $P = 0.02-0.05$.

sessed by any others of the diploids in this group, the characteristics of the polyploids can all be explained on the basis of chromosome doubling and of recombination of the characteristics of different diploids. *Crepis*, however, is a poor example from which to generalize, since the presence of apomixis would in itself tend to produce stability. More sexual polyploid complexes need to be studied from this point of view before we can tell whether *Crepis* represents the usual range of variability among the polyploid members of a complex. The sexual polyploid complex of *Paeonia* seems, according to my present knowledge (Stebbins, 1939) to hold to the same rule, in spite of the fact that it is obviously very old. In any case, however, the odds are against the polyploid complex as being the originator of any really new line of evolution. As compared with a group of diploid species, a polyploid complex tends to be a closed system.* It can produce endless new species, but these are all or nearly all new combinations of the same supply of genic material; they are new variations on an old theme. There are hundreds of species of *Rosa*, *Rubus*, *Potentilla*, *Senecio* and *Poa*, most of which have arisen by polyploidy, but the extremes of variation in these genera are no greater than they are in many other primarily diploid genera of which the total number of species is much smaller. Since the polyploid members of a complex are more numerous and wide-spread than the diploids, one would naturally expect that as a polyploid complex becomes older and as conditions cease to be favorable for the type of plant represented by that particular complex, its diploid members would be the first to go. An old or senescent polyploid complex, therefore, is one that consists only of polyploids. With increasing age, the polyploids also begin to die out, so that in the last stages of its existence a polyploid complex is simple once more, and is a monotypic or ditypic genus without any close relatives. Examples of such vestigial polyploid complexes, that is, of isolated monotypic or small genera with high chromosome numbers, are scattered throughout the plant king-

dom. Perhaps the most striking ones are the two living genera, *Psilotum* and *Tmesipteris*, which are the only survivors of the most ancient order of vascular plants, the Psilotales. Both of these genera are frequently considered to be monotypic; their species have more than a hundred chromosomes in their sporophytic cells. They may represent the remnants of polyploid complexes which flourished hundreds of millions of years ago in the Paleozoic era. We know from fossil evidence that this order formed a dominant part of the earth's vegetation at that time. Other vestigial polyploid complexes are probably the redwood, *Sequoia sempervirens*, and the genera *Lyonothamnus*, and *Fremontia*, familiar relic species of our California flora which have high chromosome numbers (Stebbins, unpub.). The evidence from the plant kingdom as a whole, therefore, suggests that polyploidy has been most important in developing large, complex and widespread genera; but that in respect to the major lines of evolution, it has been more important in preserving relics of old genera and families than in producing new ones.

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ON THE QUESTIONABLE EXISTENCE OF SEX CHROMOSOMES IN THE ANGIOSPERMS

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SWANNANOA, N. C.

THOUGH the occurrence of sex chromosomes in many species of the Angiosperms has gained almost general acceptance, there is, however, opinion to the contrary. Were we to ignore the physiological aspects of the matter, there would still be two schools of thought among cytologists. Under the pressure of genetics and its arithmetical approach much work has been contributed towards the conception that sex determination depends upon certain types of mechanisms which operate among the chromosomes during the reduction division. With one notable exception, the male plants in the Angiosperms are believed to possess the male and female determining factors and are therefore considered heterozygous to sex. Under normal conditions the pollen of such plants is of two kinds: microspores which carry the male determinant and the other half of the microspores carrying the female determinant. Numerous instances of sex chromosomes have now been described for the Angiosperms. On the other hand, some morphologists have found considerable objection to the above point of view and would include other evidence before letting the question go as a settled fact. This school of opinion is less enthusiastic about arithmetic and more critical of mechanisms than are the cyto-geneticists. Despite the extremely small chance of arriving at any compromise between these two points of view, a review of the literature on sex chromosomes in the higher plants leaves one with the impression that the issues involved in this whole problem are in a fine state of confusion. When, at this late date, the occurrence of a pair of unequal chromosomes during the first division of microsporogenesis is considered sufficient evidence in itself to claim the presence of an X-Y sex chromosome mechanism,

despite the fact that heterochromosomes are common enough among hermaphroditic plants of hybrid origin, then it is perhaps time to make a short but thorough tour of inspection.

Let us begin with a restatement of the conditions upon which the theory of chromosomal sex determination rests. As with other conventional theories on inheritance, the conception of sex chromosomes demands that one should hold rigidly to the idea that a chromosome has constant form, constant character and constant ability to impress its genetical pattern upon the ontogeny of an individual. Except for certain mechanical devices by which a degree of variation is permitted, this constancy must prevail from generation to generation, from division to division. Should the individuality of the sex chromosomes be lost but for one instant somewhere along the line, the idea of sex chromosomes must collapse. This brings us to the question of synapsis, which, in the opinion of most biologists, is no question at all. Nevertheless, it is true that the individuality of the chromosomes is preserved at a crucial stage by the idea of chromosome pairing in anticipation of the reduction division—synapsis or active pairing. The assumption of synapsis is indispensable because it is the only conceivable arithmetical arrangement whereby the reduced number of chromosomes can be derived from the diploid number and still retain the strict individuality of a specific chromosome. Were it demonstrated, for the sake of making the point, that the premeiotic chromosomes do not exist in a free state until late in the prophase, synapsis would come close to being an engineering impossibility. What then of pairing and the dependent individuality of the chromosomes, and what of the chromosome theory of inheritance itself? Under such circumstances the idea of sex chromosomes would be ridiculous. Elaboration of this contention will appear in a later paragraph.

Assuming that all is well with the individuality, the specificity and the synaptic pairing of chromosomes in

general, we must make other conditions. It is necessary to supply a reasonable mode of origin for these sex chromosomes. Since there can hardly be sex chromosomes in a hermaphroditic individual, and since the unisexual types seem to have arisen out of the hermaphroditic state, how then have sex chromosomes developed? Though complete substantiation might not be necessary, at least a rather good hypothesis for their origin would be desirable.

Furthermore, it seems necessary to demand that the phenomena now given credit as being sex chromosomes can not be essentially duplicated among the chromosomes of hermaphroditic organisms. That is, there must be something unique about sex chromosomes, other than purely theoretical imputation.

At least two other things need explanation. The first is the occurrence of intersexes, hermaphrodite or monoecious individuals, among members of an otherwise dioecious species for which sex chromosomes have been described. The second is the reported abnormalities in the actual sex-determining mechanism itself. For example, the female has the smaller number of chromosomes in *Rumex acetosa*, while in *R. acetosella* the reverse is generally true. Furthermore, the mechanisms do not operate with anything near 100 per cent. efficiency. Just what connection do such anomalies have with the organic disposition of the specific plant? For these too one ought to receive a reasonable answer.

Last, but not least, one must be very certain that there is no other explanation of the phenomena now called sex chromosomes which might have as much or more basis in fact. From the genetical point of view it might appear quite logical that there should be such things as sex chromosomes. However, such an argument is not likely to impress any one except those already prejudiced in favor of their occurrence. Is it not possible that the transfer of these genetical values, called factors or units, from the purely mathematical realm to a specific portion of an organic structure (such as a chromosome) may entail a longer jump than is now commonly contemplated?

We have now set before us a list of the principal conditions which, in the opinion of the author, the description of sex chromosomes ought to meet.

1. Individuality and specificity of chromosomes.
2. The continued success of the synaptic theory.
3. A reasonable mode of origin for sex chromosomes.
4. A unique apparatus among all types of chromosomes.
5. A good explanation of intersexes.
6. A good explanation for the frequent misbehavior of the sex chromosome mechanism.
7. Certainty that the facts can not receive a still better interpretation other than calling them sex chromosomes.

Perhaps a few words concerning the mechanical conception of sex determination should be inserted at this time. The ideas about sex chromosomes have undergone considerable change in an effort to meet the criticism of the physiologists and many experimental morphologists. From the early idea of sex being determined by a distinct chromosome which contained a single unit of inheritance for sex, the current opinions allow the interplay of numerous units so that the resulting sexuality of an individual is more of a dominance of one sexual tendency over the other. That is to say, an organism is more female than it is male, and *vice versa*. Though such maneuvering has largely satisfied the earlier criticism, the essential idea of the theory has been maintained, namely, the determination of sex depends in the last analysis upon the distribution, random or otherwise, of certain chromosome structures. No matter how much removed from the original idea of a single unit of sexual inheritance, the theory of sex chromosomes still depends upon a mechanism for its successful operation. Though an opinion on this question when brought down to its simplest terms is likely to be philosophical rather than biological, we face a choice, and our subsequent biological discipline will reflect that choice. We either look upon the chromosomes as the basic substance out of which the manifestations and organic development of life depend or we believe that the chromosomes were among the earlier structural differentiations

of the protoplasm. Many of us have been taught that organic structures have always arisen as the result of some previous action within a still more basic substance. The present theories of chromosome inheritance would largely deny this premise. In fact, they must deny it, since their assumption places the chromosome as the residence of at least the controlling factors if not the basic powers themselves. The chromosome theory of inheritance has developed elaborate schemes to explain the change which takes place in the transfer of inheritance from one chromosome to another. This too is mechanical. Though not essential to the argument of this paper, the analysis of the predicament in which we find ourselves when trying to untangle the logic behind much that has become traditional theory may have its place after all. It seems likely that one is either content or critical of the mechanical conception of organic evolution. Not that being mechanical either adds or detracts from its soundness—but simply that the chromosome theory of inheritance, including sex chromosomes, is based upon a mechanical conception of events and must therefore defend itself upon that foundation.

To examine the chromosome theory of inheritance and its relation to the description of sex chromosomes under each of the seven objective conditions just listed would call for more words and a more secure knowledge of a wealth of material than supposed authorities on the subject have apparently cared to assume. Moreover, the author has delivered himself on several of those points in other papers. The focus of this paper will dwell on points 2 and 7, namely, the continued success of the synaptic theory and the presentation of what the author believes to be a more inclusive explanation of the phenomena now called sex chromosomes.

I. SEX CHROMOSOMES AND SYNAPSIS

Since it is perfectly obvious how essential the synaptic theory is to any consideration of sex chromosomes, the

author has been careful to examine the origin of the meiotic chromosomes in a number of unisexual species for which such sex chromosomes have been described. Without exception, the premeiotic spireme is fundamentally continuous in *Rumex acetosa*, *Rumex acetosella*, *Lychnis alba*, *Smilax glauca*, *Smilax rotundifolia*, *Dioscorea quaternata*, *Ilex opaca*, as well as in numerous other dioecious and perfect species of the Angiosperms (cf. 1936a and 1937). This seems to have been Strasburger's early conception and may still be noticed in several drawings in the later editions of his text-book. Jeffrey (1937) has revived this idea and has lately (Jeffrey and Haertl, 1938) declared his opposition to the synaptic theory of meiosis. The accompanying drawing of premeiotic spireme in a P.M.C. of *Smilax rotundifolia* is fairly typical of similar stages in the above-named plants. This species of *Smilax* in the vicinity of Swannanoa and Black Mountain has 16— n chromosomes. If, by conventional theories, there are 32— $2n$ chromatids, and each chromatid has two ends (and it can not have less), then each nucleus at this stage

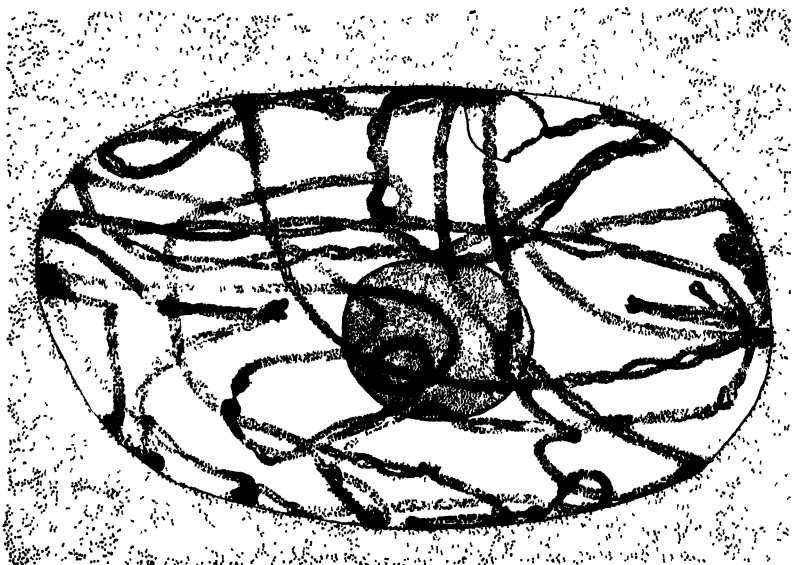


FIG. 1

should have 64 *free ends* visible. The nucleus shown on this page has but one free end visible. Certainly out of a possible 64 at least a dozen free ends would not be too much to ask. But they are simply not there, because the spireme is continuous to the full of its length. The nuclei in the reproductive tissue of *Smilax rotundifolia* are small in comparison with those of *Trillium*, *Allium*, *Lilium*, etc., and consequently not a great deal could be seen as to the spiral composition of the spireme other than it is composed of two principal "threads" whose gyres twist in opposite directions. In a few places along the spireme where its member "threads" are more distinctly separated there is evidence that each "thread" is in turn composed of two "threads," also spiral wound about each other in opposite directions. Where, then, are the individual chromosomes in the diploid number? For the sake of brevity one might advance the notion that each principal thread of the spireme represents sixteen somatic chromosomes joined end to end, and that this thread has "paired" with a similar string of sixteen somatic chromosome mates. For such a claim it is necessary to assume that the individual chromosomes became oriented into the exact position of order in each string and got its right end attached to the correct end of its anterior or posterior neighbor and that when the two strings began to pair the right end sought out the corresponding end of the sister thread. All this is hard to imagine, particularly since the double condition of the spireme is visible almost as soon as any definite structure can be discerned in the nucleus, even at the supposed "leptotene stage." In other words, synapsis in these species approaches an engineering impossibility. Rather does it seem probable that the haploid number of chromosomes becomes segmented out of a fundamentally continuous spireme, the two threads of which originate by splitting of an earlier thread. The separation of the meiotic chromosomes out of the spireme occurs more quickly in some species than in others so that the individual "synaptic pairs" exist some time before diaki-

nesis. On the other hand, particularly in hybrids, the old spireme connections between the haploid chromosomes persist even into the metaphase and anaphase of the heterotypic division (J., 1936a). Sometimes in *Smilax rotundifolia*, the otherwise continuous spireme would show a few breaks in its continuity, but in no instance did the number of *free ends* exceed half a dozen. A word about technique ought to be inserted at this time. The greatest care is necessary in order that the actual conditions of the nuclei may be preserved. Several fluids were used, including Carnoy's, with rapid penetration being insured by the use of a vacuum pump upon immersion. The material was then imbedded according to the Jeffrey (1928) hot nitrocellulose method. In the case of *S. rotundifolia* the use of hydrogen peroxide was not necessary. The sections were left in iron alum for two hours and in Heidenhain's haematoxylin for twenty-four hours. The decoloring process was done very slowly to get the maximum differentiation. In studying these prophases a superficial glance can be wildly misleading. Often the loops of the spireme are so bent that one half of the loop is optically covered by the other half. Several such loops are visible in the drawing. One is first tempted to consider such a loop as a free end of a single pair of chromatids. Nevertheless careful study, in a manner which no photomicrograph can show, will usually reveal that what may appear on first sight to be distinct chromosomes are actually portions of a fundamentally continuous spireme.

Consequent to these studies, the author can not subscribe to the theory of synapsis or active pairing of reproductive chromosomes. The behavior of the premeiotic chromosomes suggests an origin hardly favorable to the present insistence on the individuality of the chromosomes, and, as a consequence, make the support of the sex chromosome idea quite untenable. Few there are who will welcome such a conclusion and yet, if the tremendous possibilities of W. D. Francis's (1939) opinion on "The Spiral Structure of Protoplasm" eventually find applica-

tion in a similar crystalline relationship between cytoplasm and chromatin, then our present ideas on chromosomes are in for considerable modification anyway; all of which may prove very good for cytology.

II. A SUGGESTED EXPLANATION OF THE PHENOMENA GENERALLY CLASSIFIED AS SEX CHROMOSOMES

In an effort to present what the author believes to be a better explanation of the cytological phenomena hitherto called sex chromosomes, it is necessary to introduce further evidence. The first is a brief review of the systematic position of the various Angiosperms for which sex chromosomes have been described. It is quite possible that some papers describing sex chromosomes have escaped the author's attention, and knowledge of such omissions will be appreciated. Though the accompanying compilation may not be complete, the fifty species which are listed undoubtedly include the large majority of plants under discussion and certainly include all the better known or "classical" cases.

A glance at the lists will show that out of forty-nine species and one variety only the two species of *Humulus* belong to a family all members of which are strictly dioecious. However, the system of Engler and Diels was followed in citing the family. Had the system used in Gray's Manual been followed, *Humulus* would be included in the *Urticaceae*. In that case, not one would belong to a family all the members of which are strictly dioecious. Ten out of fifty belong to strictly dioecious sub-families. Twelve belong to strictly dioecious genera. The species of *Salix* and *Populus* can not be included as of strictly dioecious genera, even though they are often thought of as such. As indicated, *Salix Medemii* is strictly a monoecious species according to Engler and Diels (1936). Professor M. L. Fernald, of Harvard University, has advised me that numerous instances have been reported of varying sexual conditions in the flowers of these two genera. Perfect flowers have been noted in *Salix aurita* L., *S. fra-*

gilis, L., *S. amygdaloides* Anderss. and other Old World species. Cases of perfect flowers in *Populus monolifera* and *P. tremula* have been recorded. However, it is not just a case of perfect-flowered intersexes, because many gradations have been observed from perfect, to flowers of both sexes in the same ament, to monoecious and polygamous, as well as the more common dioecious state. Cases where carpels have changed into stamens and vice versa are also well known. It therefore seems obvious that the species of *Salix* and *Populus* are generally in a state of flux and have not established a definitely unisexual condition as yet.

Species 1 to 17 plus Nos. 48 and 49 are characteristic as a group in that they belong to genera containing both hermaphroditic as well as dioecious species (we could also include the species of *Salix*). This would indicate that the unisexual condition of these nineteen species is of particularly recent origin. This group includes the "classical cases" of sex chromosomes ascribed to *Rumex*, *Lychnis* (*Melandrium*) and *Helodea* (*Elodea*). These facts would seem to add up to the opinion that almost all the Angiosperms for which sex chromosomes have been described belong to groups which still show considerable instability as to sexual differentiation and for the most part have only recently acquired the unisexual habit.

It is also of some interest to notice that thirty-nine of the fifty species belong to genera having thirty or more species. Eight out of fifty belong to genera having less than ten species. No species in the list is monotypic. Though the genus *Fragaria* is only given eight species, numerous varieties exist for some of these species, especially *F. virginiana*, some of them being interspecific hybrids.

Though the numerical quantity of forms within a certain genus can not accurately be compared with the number within another genus, since this number depends so often upon the degree to which varieties are given species standing, it is nevertheless obvious that these plants which

are supposed to have sex chromosomes belong to genera of considerable size. Perhaps the diversity of sexual habit and relatively large number of forms found in these genera appear to have little bearing on the discussion. However, these two tendencies may have significance when the cytological condition of these genera is examined more closely. The occurrence of hybrids and varieties has already been mentioned for *Fragaria*. The author's investigation of the genus *Rumex* (J., 1936a) give ample evidence that the extreme polyploidy in the Eulapatham section of the genus is the direct result of interspecific hybridization and, though polyploidy in the Acetosa section has been more restricted, many of the species still possess meiotic irregularities diagnostic of a hybrid ancestry. Various chromosome numbers occur within a single species. For example, *Rumex acetosa* has at various times been found to have 14, 15, 16, 17, 20, 21, 22 and 29 diploid chromosomes. The literature is filled with cases of natural hybrids in this genus. Even with a loose standard, this is hardly a stable condition; yet for *R. acetosa* more effort has gone into the description and study of the sex chromosomes than any other species of higher plants.

The genus *Lychnis* has not given much evidence of polyploidy so far, but it is common knowledge among horticulturists that numerous garden varieties and hybrids exist for several species. The author found that the hermaphroditic *Lychnis chalcedonica* possessed a normal reproductive process, while the dioecious *L. alba*, for which sex chromosomes have been described, presented unmistakable evidence of a hybrid ancestry. The meiotic process displayed lagging chromosomes, chromosome fragmentation, extruded chromatin, occasional extra nuclei and abortive spores. The degree of pollen sterility in some anther sacs was very high. These are characteristic phenomena of known hybrids in contrast with the regular behavior of the parents (J., 1939) and without involving the so-called X-Y apparatus itself bespeak a hybrid ancestry not too distantly removed for *L. alba*.

No.	Name of plant	Authority for sex chromosomes	Family name	Sexual condition of family	Sexual condition of sub-family	Sexual condition of genus	No. of species in genus	Other notes
1.	<i>Rumex acetosa</i>	Kihara and Ono	Polygonaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	100	mMm complex
2.	<i>R. acetosella</i>	Meurman	"	"	"	"	"	"
3.	<i>R. thyrsiflorus</i>	"	"	"	"	"	"	"
4.	<i>R. arifolius</i>	Kihara and Ono	"	"	"	"	"	"
5.	<i>R. nivialis</i>	"	"	"	"	"	"	"
6.	<i>R. montanus</i>	Kihara and Yamamoto	"	"	"	"	"	"
7.	<i>R. hispanicus</i>	Jaretsky	"	"	"	"	"	"
8.	<i>Lycchnis alba</i>	Blackburn, Winge, Heitz	Caryophyllaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	90	X-Y complex
9.	<i>L. dioica</i>	Blackburn	"	"	"	"	"	"
10.	<i>L. discricata</i>	"	"	"	"	"	"	"
11.	<i>L. platinosa</i>	"	"	"	"	"	"	"
12.	<i>L. rubra</i>	Meurman, Heitz	"	"	"	"	"	"
13.	<i>Silene alites</i>	Blackburn	"	"	"	"	"	"
14.	<i>Valeriana dioica</i>	Meurman	Valerianaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	400	X-Y complex
15.	<i>Prunella elatior</i>	Kihara	Rosaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	200 plus	"
16.	<i>Xanthoxylum piperitum</i>	Nakajima	Rutaceae	Herm.—Di.	Herm.—Di.	Herm.—Di.	8	"
17.	<i>Helecia canadensis</i>	Santos	Hydrocharitaceae	Herm.—Di.	Herm.—Di.	Polyg.—Di.	15	♀ heterozygous
18.	<i>Valisneria spiralis</i>	Winge	"	Herm.—Di.	Herm.—Di.	Herm.—Polyg.—Di.	8	Small says 150 sps.
19.	<i>Euchlaena joan</i>	Nakajima	Santalaceae	Herm.—Di.	Herm.—Di.	Di.	2	"
20.	<i>Urtica dioica</i>	Meurman	Urticaceae	Herm.—Di.	Polyg.—Di.	Di.	4	"
21.	<i>Empetrum nigrum</i>	Hagerup	Empetraceae	Mon.—Polyg.—Di.	Polyg.—Di.	Polyg.	30	"
22.	<i>E. hermaphroditum</i>	"	"	"	"	"	3	"
23.	<i>Smilax hederacea</i>	Nakajima	Liliaceae	Herm.—Di.	Di.	Di.	300	No s.x. in some sps.
24.	<i>S. Oldhami</i>	"	"	"	"	"	"	"
25.	<i>S. China</i>	"	"	"	"	"	"	"
26.	<i>Dioscorea gracilima</i>	Meurman	Dioscoreaceae	Herm.—Di.	Di.	Di.	600	No s.x. found in <i>D. quaternata</i>
27.	<i>D. sinuata</i>	"	"	"	"	"	"	Numerous instances
28.	<i>Populus trichocarpa</i>	"	Salicaceae	Mon.—Di.	Mon.—Di.	Di.	30	"

No.	Name of plant	Authority for sex chromosomes	Family name	Sexual condition of family	Sexual condition of sub-family	Sexual condition of genus	No. of species in genus	Other notes
29.	<i>P. bakamifera</i>	Meurman	Salicaceae	Mon.—Di.	Mon.—Di.	Di.	30	Numerous instances of perfect flowers
30.	<i>P. Simoni</i>	"	"	"	"	"	"	
31.	<i>P. Eugeniai</i>	Blackburn	"	"	"	"	"	
32.	<i>P. generosa</i>	"	"	"	"	"	"	
33.	<i>P. serotina</i>	"	"	"	"	"	"	
34.	<i>P. tremuloides</i>	Blackburn and Harrison	"	"	"	"	"	
35.	<i>P. Sieboldii</i>	Nakajima	"	"	"	"	"	
36.	<i>Salix Anderssonia</i>	Blackburn and Harrison	"	Mon.—Di.	Mon.—Di.	Mon.—Di.	170	<i>Salix Medemii</i> strictly mon.
37.	<i>S. aurita</i>	"	"	"	"	"	"	
38.	<i>S. Bakko</i>	Nakajima	"	"	"	"	"	
39.	<i>S. Caprea</i>	"	"	"	"	"	"	
40.	<i>S. grandulosa</i>	"	"	"	"	"	"	
41.	<i>S. babylonica</i>	"	"	"	"	"	"	
42.	<i>S. integra</i>	"	"	"	"	"	"	
43.	<i>S. sachalinensis</i>	Kihara, Nakajima	"	"	"	"	"	
44.	<i>Humulus lupulus</i>	Winge	Moraceae	"	Di.	Di.	2	
45.	<i>H. japonicus</i>	"	"	"	"	"	"	
46.	<i>H. lupulus</i> var. <i>Cordifolius</i>	Nakajima	"	"	"	"	"	
47.	<i>Cocculus trilobus</i>	"	Menispermaceae	Di. (almost always)	Di	Di.	11	
48.	<i>Trichosanthes japonica</i>	"	Cucurbitaceae	Herm.—Mon.—Di.	Mon.—Polyg.—Di.	Mon.—Di.	50	
49.	<i>T. cucumeroides</i>	"	"	"	"	"	"	
50.	<i>Dioscorea Tokoro</i>	"	Dioscoreaceae	Herm.—Di	Di.	Di.	600	

Note. The systematic nomenclature taken from Engler-Deils "Syllabus der Pflanzenfamilien," 11th ed., 1938, Gebrüder Borntraeger, Berlin.
The above table has been compiled from the lists and references given by Gaiser (5), Darlington (2), Sharp (15), and Nakajima (14).

Key to abbreviations

Herm. Hermaphrodite
Mon. Monocleous
Polyg. Polygamous
Di. Dioceous
s.x. Sex chromosomes

In his recent paper Nakajima (1937) reports the presence of laggards among the meiotic chromosomes of the following species, for which he also described sex chromosomes. *Cocculus trilobus*, *Xanthoxylum piperitum* (also the presence of a univalent, small in Nakajima's material, large in Sinoto's material; also premature migration of some chromosomes), *Smilax Oldhami* and *Smilax China*. The author has investigated two native species of *Smilax*, *S. glauca* and *S. rotundifolia* (J., 1937). Meiosis in *S. glauca* takes place without any visible abnormalities in microsporogenesis which could be even vaguely interpreted as heterochromosomes. In *S. rotundifolia* meiotic irregularities do occur in microsporogenesis, just as in most hybrids or species of hybrid origin, but the irregular behavior of certain chromosomes could hardly be allied with sex determination. A numerical difference in the chromosome complements of the four microspores did exist as a result of an abnormal type of reduction division in approximately two thirds of the P.M.C.'s. The remaining third behaved in the normal manner. However, so many other peculiarities were associated with these divisions that it was difficult to conceive of any sex chromosome mechanism having been developed. From the descriptions of meiosis by Nakajima and the author's own investigations it appears that the genus *Smilax* is composed of species of varying cytological disposition; some of which are definitely not far from previous hybridization.

Though most of the six hundred species of *Dioscorea* are found in tropical regions, a few are found further north. Of these *D. quaternata* (Walt) Gmel. was found to possess an odd chromosome which misbehaved in about a third of the P.M.C.'s. It sometimes took on the appearance of a heterochromosome pair, and sometimes split into three fragments. However, there was not sufficient regularity of behavior or the desirable nuclear deficiency to dare consider such a feature a sex chromosome complex. To consider the abnormalities rather as vestiges of

hybrid induced irregularities seemed more compatible with the facts.

Tippo (1938) has gathered the chromosome numbers of the *Moraceae* and presents the following list: 8, 9, 10, 12, 13, 14, 15, 16, and 28. For the *Urticaceae* 7, 12, 13, 14, 16, and 24. According to Gaiser (1930) *Populus* has numbers of 4 and 19, *Salix* 19, 22, 38 and 57. Woodworth (1929) came to the conclusion from his studies on the *Betulaceae* that dysploidy, such as in *Moraceae*, *Urticaceae* and *Salicaceae*, is the result of meiotic irregularities instigated by interspecific hybridization. Therefore the existence of this uneven form of polyploidy, dysploidy, in the three families under discussion is significant in that the abnormalities which gave rise to such odd haploid numbers may have been responsible also for the occurrence of these so-called sex chromosomes.

Consequent to this study of the fifty Angiosperms which are supposed to have sex chromosomes, we might summarize the results, as follows.

The best possible species in which to search for sex chromosomes should have the following characteristics.

1. The species should belong to a large and variable genus.
2. The species should belong to a genus in which both perfect and dioecious species occur.
3. The species should belong to a genus wherein the species display frequent modifications or reversals of the general sexual habit.

Warning! Avoid investigating monotypic species which give no sign of variation.

This summary might be rewritten to read: the phenomena interpreted as sex chromosomes are most likely to appear among species subject to previous hybridization and in which the unisexual habit is of comparatively recent acquisition and as yet not completely stabilized.

The author has been forced to take the position that the plants possessing certain cytological phenomena hitherto called sex chromosomes belong to that innumerable host, plants of hybrid ancestry. Since it is implied that hybridization has been the agent whereby these meiotic irregularities, along with many other types of chromo-

some behavior, have arisen, we need must answer one specific criticism. It is necessary to explain the constant difference in the chromosome complement of the male and the female plants of a species possessing sex chromosomes. For example, if they are not sex chromosomes, why do the female plants of *Rumex acetosa* have fourteen diploid chromosomes while the male plants have fifteen chromosomes, generation after generation? In this case there is not only a recognizable difference between the supposed male and female determining chromosomes, but a numerical difference in the complements as well. From a morphological point of view this argument is the soundest bulwark in the sex chromosome idea. Here at least there is nothing vague or statistical. Yet there is an answer.

In the first place, the numerical difference is not always constant. Witness the male plants of *Rumex acetosa* with 15, 16, 17, 20, 21, 22 and 29 diploid chromosomes. This variability is also true of the structural difference in the heterochromosomes or sex chromosome complex of *Rumex acetosa*, *R. acetosella* and *Lychnis alba* (J., 1936a and 1936b). Nevertheless, despite any vagaries which might occur among the so-called sex chromosomes, it is evident that there is at least a *constant tendency* toward the maintenance of these visible differences in the chromosomes of the two sexes. For this too there is an explanation built upon the knowledge of hybrid cytology. Let us go back for a moment to the circumstances surrounding the origin of the dioecious condition itself.

Suppose that a certain race of plants, lately hybridized, gradually developed from the hermaphroditic to the unisexual condition. The advent of the unisexual state introduces a new situation in the race. Each act of fertilization actually becomes an act of hybridization, since no two individuals of this hybrid race are likely to have exactly the same genetical composition. Unlike a hermaphroditic race wherein the chances are best for fertilization of an egg by sperm originating from spores of the same plant

as the egg (making proper exception for cases of self-sterility or unusual floral mechanics), the dioecious plant broadens the base for competition for the egg. The sperm must now come from some other plant, and might just as well be from a plant of the same species or some related and compatible variety, species or genus. Thus the unisexual conditions insures the constant introduction of "alien blood." This arrangement also amounts to a postponement of the normal progress of plants of hybrid origin towards the establishment of completely normal meiosis. We can therefore expect to find that such meiotic disturbances which have originated through interspecific hybridization are much more likely to persist in the plants which take on the unisexual habit than in those which remain hermaphroditic or monoecious. The genus *Rumex* exhibits this in a remarkable manner. The *Eulatham* section of the genus is highly polyploid, hermaphroditic and prone to hybridize in nature. Yet the regular species are inclined to be normal in their meiosis (J., 1936a and 1936b). On the other hand, the *Acetosa* section is but irregularly polyploid (dysploid), dioecious and seldom hybridizes in nature. Yet almost all the species of this section which have been investigated display numerous meiotic abnormalities reminiscent of the natural hybrids found among the species of the *Eulatham* section. To the evidence that these plants for which sex chromosomes have been described are of hybrid origin and decidedly unstable as to sexual condition we may now add the conclusion described above that the dioecious condition introduces a circumstance by which this instability is likely to survive for a much longer time than in the hermaphrodite forms.

With the previous paragraph as a background, we may now proceed to a closer scrutiny of the actual relationship thought to exist between the sexes. In conventional terms this is described as being either homozygous or heterozygous to sex. That is, one sex of a given species contains determinants for both sexes of the offspring, and

is therefore heterozygous to sex. The opposite sex, however, is assumed to contain only the determinants for its own sex and is therefore homozygous to sex. This definition may ignore some of the niceties which some might wish added, but probably includes the principal distinctions. With the exception of *Fragaria elatior*, Kihara (1930), the male has been found to be heterozygous to sex in the Angiosperms. It is along with this heterozygosis that the cytological phenomena of sex chromosomes appear; either the numerical or heterochromosome differentiation in relation to sex determination occurs in but one sex. We must remember that from a *morphological* point of view the XX "pair" which appears in the reduction division of the megaspore mother cell of all these species with the exception of *Fragaria elatior* behaves and looks just like any other "pair" of autosomes. Only by the supposed association of an X with a Y chromosome (nM with M or nN with n) does the female sex chromosome or the chromosome for the homozygous sex become unique among the chromosomes of that sex. Consequently, if as in almost all the Angiosperms it is the male which reveals the meiotic peculiarity called heterochromosomes of sex, the remaining problem is simply to suggest a sound reason why this tendency to abnormal meiosis persistently appears in one sex.

The assumption that one sex was strictly the heterozygous one occurred more than a decade before the first sex chromosomes were described. Strasburger (1910) and Correns (1922) explained the inequality in the number of male and female plants in natural populations by assuming that the male was heterozygous and, though the pollen was probably half male and half female determining in nature, there was a competition between the pollen tubes of the two sorts of spores. Thus, a population in which the males outnumbered the female plants in a three to one ratio was due to the stronger or more rapidly growing tubes of the spores carrying the male determinants. For the most part these ideas were carried over into the

description of sex chromosomes. Here is the crux of the whole matter—because the reproductive cells of one sex were demonstrably variable as to sexual disposition (male or female inclined), it has been assumed that the opposite sex was constant in its sexual disposition, or homozygous. The author challenges that contention. Since these plants for which sex chromosomes have been described are definitely in a state of sexual instability and give every evidence of being rife with hybrid abnormalities among the chromosomes as well as in variability of habit, it seems difficult to subscribe to the idea that the female is anywhere near homozygous to sex. In the face of everything we know about the variable condition of hybrids, it seems more logical to assume that in any given species the eggs in any given female can hardly be presumed to have exactly the same disposition towards the sexuality of the offspring which will develop from these same eggs. On the male side the most flagrant partiality is observed. Not only is the sexual potentiality of the various pollen grains thought to differ, but the ability to transmit this difference into a growth or selective factor in the pollen tubes as well. Thus the assumption of a strict heterozygous and homozygous distinction between the two sexes is the same thing as admitting that the factors which determine sex are not subject to the evolutionary laws which we have found to operate in every other phase of hybrid cytology. According to the current opinion, any change in the sexual habit must therefore occur in the heterozygous sex alone, without a contribution from the companion sex. Such an indifference upon the part of the egg in the Angiosperms would be appalling, particularly in view of the evident hybrid ancestry and sexual instability of the plants for which sex chromosomes have been described.

For the sake of completing the proposed hypothesis, let us assume that both sexes are to some extent heterozygous to sex, even though one sex (usually the male in the Angiosperms) carries a greater dominance. In that case no resort need be made to sex chromosomes for an ex-

planation. If, as in most Angiosperms, the appearance of heterochromosomes or any other peculiar type of meiotic apparatus is associated with the male, then the problem resolves itself into getting sperm, possessing this ability, to fertilize or fail to fertilize the egg less disposed or more disposed to femaleness respectively. Let us use *Lychnis alba* to demonstrate this point. In this species the male is thought to be "heterozygous" and consequently it is in this sex that the heterochromosomes are found. The female possesses an XX "pair" at the time of the reduction division; the divisions are regular; the chromosome complements of the megaspores can not be distinguished. Since *L. alba* gives definite evidence of previous hybridization and its systematic proximity to hermaphroditic species suggest the recent origin of the unisexual condition in this species, it is quite probable that the eggs are variously inclined, some being more strongly female than others. Since regular meiosis is associated with the female side, those eggs which are more strongly female in tendency will naturally find a more compatible match in such sperm as have the full and complementing set of chromosomes. On the other hand, such eggs as are weaker in their sexual tendency to femaleness will find the sperm possessing the deficient complements more acceptable. Hence it follows that though sex is definitely linked with peculiar meiotic phenomena in some species of Angiosperms, the occurrence of the same seems to have no bearing whatsoever on the determination of the sex of the offspring. The principal errors which have led to the assumption of sex chromosomes appear to be the lack of appreciation of the role played by hybridization in species evolution, confidence in the implications of the terms "homozygous" and "heterozygous" and lack of familiarity with the behavior of known hybrids. The author is in no way prepared to give an opinion as to just how much variability exists on the female side of these unisexual forms. Experience with hybrids would suggest that it might be somewhat less on the female side than on the

male. On the other hand, the case of *Fragaria elatior*, already mentioned, suggests that in some cases the female might carry the tendency to exceed the male in meiotic abnormalities. Probably intermediate stages exist between the *Lychnis* type and the *Fragaria* type. It is freely admitted that nothing has been stated concerning the reason for the association of these peculiar meiotic abnormalities with a particular sex. Täckholm (1922) discovered a difference in meiotic behavior in microsporogenesis and megasporogenesis of the *Canina* roses, which is also a common feature to an unpredictable extent in most interspecific hybrids of the genus *Rumex* (J., 1936a). Whatever the final interpretation may be, this point has little direct bearing on the problem under discussion.

It has been the thesis of this paper that a consistent explanation of the cytological phenomena generally interpreted as sex chromosomes could be developed which would be more in harmony with the increasing knowledge of the behavior of hybrids. Because the author has already covered some of the objections to the sex chromosome theory in other papers, they were not repeated here. In some respects it might appear that the essence of the views here expressed consist of a reversal of the common conception of events. Whereas the conventional ideas on sex chromosomes terminate with a mechanical device among the reproductive chromosomes which determines the sex of the offspring, the author's opinion holds that the determination of sex, when combined with the forces initiated by previous hybridization and the acquisition of the unisexual habit in a species, will logically produce the transient phenomenon of "sex chromosomes." It therefore follows that "sex chromosomes" have nothing to do with the actual determination of sex—they are simply one of the by-products of organic evolution.

SUMMARY

The following have been presented:

1. An analysis of the conditions upon which the theory of sex chromosomes rests.

2. Evidence against the idea of synopsis.
3. A study of the peculiar systematic and cytological characteristics of the fifty species of Angiosperms for which sex chromosomes have been described.
4. Discussion of the probable fallacy of assuming that any of these fifty species possess a sex which is completely homozygous to sex.
5. An interpretation of the phenomenon now classified as sex chromosomes which is believed to be more in keeping with the facts.

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SHORTER ARTICLES AND DISCUSSION

ADAPTATIVE DIFFERENCES IN THE EGG MEMBRANES OF FROGS

THE influence of temperature in limiting geographical distribution is apparent in many animal groups. One species will be characteristic of a region where the temperature fluctuates around a certain mean, while yet another is found under warmer or colder conditions. To throw light on some aspects of this problem a study is being made of physiological differences among members of the Salientian genus *Rana* that inhabit northeastern North America. These frogs differ in the extent of their northern distribution and, in a given locality in the time at which they spawn. It has previously been shown (Moore, 1938) that marked differences exist in the embryonic temperature tolerance, northern frogs having lower minimal and maximal temperatures for normal development. Furthermore, northern species compensate for the retarding influence of the low temperature of their environment on development, by a rapid rate of growth (Table 1). The present study is concerned with modifications of the jelly envelopes which limit the developing embryos to particular temperature conditions.

TABLE I

Species	Order of breeding	Northern limit	Embryonic temp range	Hours to stage 20 at 20° C.
<i>R. sylvatica</i>	1	67° 30' N	2.5-25° C.	72
<i>R. pipiens</i>	2	60° N	6 - 28° C.	95
<i>R. palustris</i>	3	51-55° N.	7 - 30° C.	105
<i>R. clamitans</i>	4	50° N.	11 - 35° C.	112
<i>R. catesbeiana</i>	5	47° N	15 - ?° C.	131

THE JELLY MASS OF SPRING BREEDING FROGS

The three species of frogs which breed early in the year, *Rana sylvatica* Le Conte, *Rana pipiens* Schreber and *Rana palustris* Le Conte have a similar spawn. This is in the form of an irregular oval jelly mass which is usually attached below the surface of the pond. The number of eggs varies from 700 to 900 in *Rana sylvatica*, to from 2,000 to 3,000 in *Rana pipiens*. The volume of these masses several hours after fertilization varies from 100 to 150 cc.

This type of jelly mass presents several problems connected with the exchange of gases between the embryos and the environ-

ment. The outermost eggs are separated from the surrounding water merely by the thickness of their own membranes. Those in the center of the mass, however, are some distance from the periphery (as much as two inches), and the amount of oxygen available for their needs is diminished by the respiration of the eggs nearer to the outside. However, at low temperatures the oxygen available to the inner eggs will be greatly increased as:

(1) The surrounding water will contain more dissolved oxygen leading to a greater diffusion of this gas into the egg mass.

(2) The oxygen requirements are diminished by a reduction in temperature, and those embryos near the periphery will not exhaust the available supply of this gas as rapidly as at higher temperatures.

It would seem likely, then, that the oval type of jelly mass characteristic of *Rana sylvatica*, *Rana pipiens* and *Rana palustris* is best suited to cold waters. At temperatures below 15° C. most of the eggs will hatch, although those in the center of the mass may be retarded in their development. Above this temperature the mortality increases regularly. The inability of eggs in a jelly mass to survive at moderately high temperatures is shown in the following experiments.

Eggs of *Rana sylvatica* were collected under natural conditions and brought into the laboratory for immediate use. They were in stage 14 (Pollister and Moore, 1937). The entire experiment was conducted in a room held at 25° C. Although this is supra-maximal for cleavage stages, it is within the normal temperature range for older embryos. Some of the egg masses were placed in aquaria with just enough water to cover them. They were kept in this manner to determine the behavior of entire jelly masses under temperature conditions simulating those in the environment of the summer breeding frogs to be discussed later. Other masses were divided into groups of 2-5 eggs and placed in finger-bowls. These served as a control for rate of development and temperature tolerance. If, as has been postulated, eggs in the entire mass would suffer an oxygen lack, this could be lessened by keeping an atmosphere of oxygen above them. To test this, entire masses were kept in corked 2L. flasks into which oxygen was bubbled four times daily. The results were as follows (Table 2). Eggs in the entire masses were retarded in their rate of development and finally died in stages 17 and 18. Not one tadpole hatched under these conditions. The eggs in groups of 2-5 de-

TABLE II

EXPERIMENT IN WHICH EGGS OF *RANA SYLVATICA* WERE KEPT UNDER DIFFERENT CONDITIONS. TEMPERATURE 24.1-25.0° C.

Number of experiments	Condition of eggs	Atmosphere	Result
10	In entire mass	Air	Eggs die in stage 17-18. None hatch
3	In small groups of 2-5 eggs	Air	Over 90 per cent. hatch as normal tadpoles
6	In entire mass	Oxygen	Over 90 per cent. hatch as normal tadpoles

veloped at a uniform rate and hatched into normal tadpoles. Likewise the entire masses kept under an atmosphere of oxygen hatched. Some of the eggs in the center of these bunches were slightly retarded when compared with those at the periphery, but they eventually hatched. From these experiments we may conclude that the eggs of *Rana sylvatica* (also true for *Rana pipiens* and *Rana palustris*) are not able to survive in this type of jelly mass at 25° C. Furthermore, it seems likely that the failure to survive is due to asphyxiation. Under natural conditions, however, the eggs are not exposed to high temperatures. When *Rana sylvatica* breeds, the water temperature is about 10° C. and mortality is very low. The two additional species with this type of jelly mass breed at a slightly higher temperature; *Rana pipiens* at 12° C. and *Rana palustris* at 15° C. (Wright, 1914). It is now of interest to learn if the latter two species, breeding under warmer conditions but still retaining the compact jelly mass, differ from *Rana sylvatica* in any way that may be considered as an adaptation to the increased temperature.

Such an adaptation has been found in the stage at which these embryos hatch. It will be recalled that the amount of oxygen consumed in frog embryos increases with age (Atlas, 1938). Thus the oxygen deficiency described previously would become greater and greater the longer the embryo remained in the jelly envelopes. When hatching occurs the young tadpole is in direct contact with the surrounding water. *Rana sylvatica* hatches toward the end of stage 20 and in early 21 (Fig. 1). The tadpole at this time has well-branched external gills and is capable of swimming. *Rana pipiens*, breeding under warmer conditions, hatches in stage 18 before the heart has begun to beat, but when muscular contraction is possible. *Rana palustris*, breeding under still warmer conditions, hatches in stage 17. At this time the tail-bud has just appeared, and muscular contraction after mechanical stimu-

lation is not possible. Thus we find a progressive reduction in the time the embryo remains in the jelly envelopes in species breeding under warmer conditions.

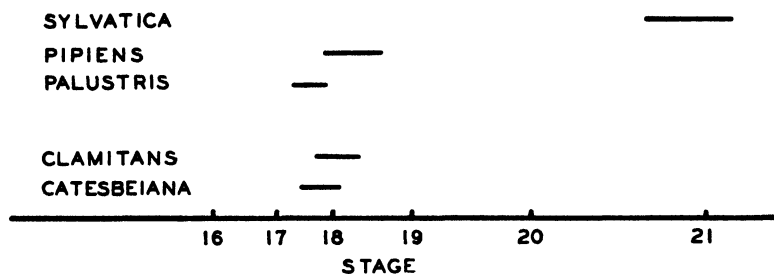


FIG. 1. The morphological stage in which hatching occurs in five members of the genus *Rana*.

THE EGG MEMBRANES OF SUMMER BREEDING FROGS

Rana clamitans Latreille breeds in June when the temperature of pond water has reached 25° C. (Wright, 1914). (We may recall that at this temperature there was no survival in egg mass of *Rana sylvatica*.) *Rana catesbeiana* Shaw breeds in July, and most likely the water temperature is slightly higher. The compact jelly mass so characteristic of the early spring breeding frogs is absent in these two species. The eggs are laid as a film that floats on the surface of the water. Instead of being closely crowded they are well separated, and are connected on but one plane. They are thus in a position to secure a better oxygen supply. An egg film of this nature would be fatal in the early spring breeders, as quite frequently a cold spell after the spawning period causes the surface of ponds to freeze. Eggs so trapped would be killed. Both *Rana clamitans* and *Rana catesbeiana* hatch at an early morphological stage (Fig. 1).

The members of the genus *Rana* employed in this study illustrate a general principle applying to many poikilothermic animal groups; namely, species differ in the temperatures to which they are best suited, as shown by their north-south distribution and their time of breeding in a given locality. In a study of the physiological factors adapting frogs to a particular geographic range and breeding season, it has previously been shown that the embryonic temperature tolerance varies to cover conditions in the environment at the time of spawning, and that northern animals offset the retarding effect of low temperature on development by a

more rapid rate of growth. In the present study it was shown how the structure of the egg mass would tend to limit these species in their breeding. Those frogs breeding early in the spring when the water is cold have a submerged compact jelly mass. The closely crowded eggs, however, die of asphyxiation at temperatures such as those existing in the environment in which *Rana clamitans* and *Rana catesbeiana* breed. The latter two species deposit their eggs in a surface film that insures a better supply of oxygen.

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CROSSING OVER AND SEX IN THE FOWL¹

THE various animals investigated have shown wide divergence regarding the influence of sex on the percentage of crossing over between genes known to be carried by the same chromosome. The extremes of reaction are shown by *Drosophila* on the one hand with crossing over normally occurring only in the female, and the silkworm on the other, with crossing over limited to the male. Other forms show the incidence of crossovers to be practically identical in the two sexes.

The fowl is of special interest, since it differs from most animals in which the phenomenon of crossing over has been investigated, in that the female is the heterogametic sex. It is only recently that sufficient data on favorable material have been accumulated to give evidence of the relationship of crossing over to sex in the fowl. Landauer (1933) and Taylor (1934) have presented a large amount of data on the linkage relations of rose comb and creeper characters in the fowl. Their results involved crossing over in both males and females, but the crossover per-

¹ Contribution No. 117 from the Department of Poultry Husbandry.

centage was so low (less than 1 per cent.) that the data were not favorable for determining the effect of sex on the incidence of crossing over. Considerable data have been published on the linkage relation of sex-linked factors in the fowl, but such data are of no value for establishing the relationship of sex to the phenomenon of crossing over.

TABLE I
EFFECT OF SEX ON CROSSING OVER IN THE FOWL

	Crossing over in females			Crossing over in males		
	Total gametes	No. cross overs	Per cent. crossing over	Total gametes	No. cross overs	Per cent. crossing over
Crest—Dominant White						
Warren	1,268	160	12.3	2,278	280	12.3
Hutt	35	2	5.7	379	55	14.5
Total	1,303	162	12.4 \pm 0.62	2,657	335	12.6 \pm 0.43
Frizzling—Dominant White						
Warren	315	62	19.7	1,222	224	18.3
Hutt	248	46	18.5	337	48	14.2
Total	563	108	19.2 \pm 1.12	1,559	272	17.4 \pm 0.64
Frizzling—Crest						
Warren	584	173	29.6	1,087	301	27.7
Hutt	35	11	31.4	80	24	30.0
Suttle & Sipe	235	68	28.9	81	22	27.2
Total	854	252	29.5 \pm 1.05	1,248	347	27.8 \pm 0.85
Silkie—Flightless						
Warren	1,079	125	11.6 \pm 0.66	580	71	12.2 \pm 0.92
Rose Comb—Creepers						
Landauer	4,313	23	0.53 \pm 0.074	3,095	5	0.16 \pm 0.048
Taylor	683	4	0.59 \pm 0.197	1,500	6	0.40 \pm 0.109
Total	4,996	27	0.54 \pm 0.070	4,595	11	0.24 \pm 0.049

In Table 1 are data on autosomal linkage in the fowl. Both the published data of various workers and previously unpublished results of the author are included. The data from all sources have been combined for the comparison of the crossover percentages in the two sexes and involve 5 pairs of characters. Both the coupling and repulsion phases are included in the totals given for each pair of characters. A very large percentage of the results is from one-year-old birds. The data credited to Hutt are those reported by Warren and Hutt (1936); and those of the author include those given in that publication combined with a considerable body of recently acquired data.

The data in Table 1 show very similar percentages of crossing over in males and females. In no instance is the difference between the sexes more than 2 per cent. The numbers involved are fairly large, and in only one case (rose comb—creepers) is the difference between males and females statistically significant as indicated by the probable errors of the means. In the five tests

reported the females twice slightly exceeded the males in percentage of crossing over, while in the other three, the males slightly exceeded the females. It would seem, then, that the phenomenon of crossing over is very little, if at all, influenced by sex in the fowl. Any existing effect of sex is so slight as to be negligible in the determination of linkage relations in this form.

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ANOTHER NEW YORK RECORD FOR THE FRESH-WATER MEDUSA

THE fresh-water medusa, *Craspedacusta sycderi*, generally believed to be rare, is reported from a few new localities each year. I should like to report its occurrence in Garnet Lake, Warren County, New York. The medusae were seen swimming in the lake on September 7 and 8, 1938. On September 9 about one hundred specimens were taken and preserved. This number was easily taken, for, as other collectors have found, the medusae often occur by the thousands. The medusae, never before reported from this lake, were found in two areas, separated from each other by over a mile of open water.

Garnet Lake is located about fifteen miles south of North Creek, N. Y. On some maps it is also listed as Mill Creek-Pond or Mill Pond. However, the pond was greatly enlarged in 1851 by the construction of a dam across the outlet, and since then it has usually been called Garnet Lake. It is now about two miles long and varies in width from one fourth to one half of a mile. About one half of the lake is very shallow and is filled with decaying tree trunks, which were inundated when the dam was built. The medusae, however, were all found in the open end of the lake.

All the Garnet Lake medusae examined were females. Their ovaries were filled with eggs which were apparently in late stages of oogenesis. Dr. Dayton Stoner, of the New York State Museum, very kindly sent me some medusae which he collected during the summer of 1937 at Loudonville, N. Y.⁴ When gonads of these were sectioned it was found that they were all males still in active stages of spermatogenesis. Spermatozoa were plentiful in all gonads, as were spermatids and groups of dividing spermatocysts. A comparison of the gross morphology of these cytologically determined males with cytologically determined females showed that the sexes are similar in all characteristics except the shape of the gonads. The female gonad is roughly oval or bean-shaped. The male gonad is of the same general shape, except that the distal end is greatly elongated and, therefore, hangs farther down into the sub-umbrella cavity than does the female gonad.

The distinction between the American species of the fresh-water medusa and the European species is not as yet very clear. Although elucidation of all the peculiarities of the two forms must await a complete study of the life histories of both species, the following observation may prove of interest. A comparison of the Garnet Lake medusae with topotypes of *Craspedacusta sowerbii*, loaned to me by the British Museum of Natural History, showed these two forms to be essentially similar except for the size of the tentacles. It was found that tentacles of the same length were much more slender in *C. sowerbii* than in *C. ryderi*. This difference does not seem to me to have resulted from the methods of preservation.

Craspedacusta has previously been reported for New York State by Breder from the New York Aquarium;¹ by Robertson from Lackawanna;² by Davis from Staten Island;³ and by Stoner from Loudonville.⁴

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¹ *Science*, 67: 242, 1928.

² *Science*, 80: 403, 1934.

³ *Proc. Staten Is. Inst.*, 8: 15-16.

⁴ *Science*, 87: 188-189, 1938.

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THE AMERICAN SOCIETY OF NATURALISTS 'CELL REACTIONS'

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It was my first thought to speak on cell behavior, but it becomes immediately evident that behavior results only from reactions in orderly sequence in time and space. The reactions themselves have long been within the technical range of the experimental biologist, and there has even been the beginning of an approach to the study of the orderly sequence that results in patterns both in organization and in behavior. The discovery of organizers gives a welcome glimpse into a world of mystery, just as the earlier studies on hormones threw some light on the machinery of the apparently incomprehensible harmony in bodily relations and behavior of the parts of the individual.

That single cells take an active but apparently regulated and controlled part in carrying on the bodily differentiation and activities of the individual; that such cells stand in somewhat the same relation to the life of the individual as the differentiated members to a community such as that maintained by certain social insects; that their activities create survival values not for themselves but for the individual of which they form a part—these propositions constitute my subject to-night. A few examples, familiar enough no doubt to most of you, will illustrate my meaning.

For my purpose, cell reactions may be considered to be intra-cellular, intra-organismal or extra-organismal. Of

¹ Address of retiring president, American Society of Naturalists, Columbus, Ohio, December 30, 1939.

reaction following an intra-cellular stimulus I will cite one example.

In the red alga *Agardhiella* the egg cell is very small and poor in cytoplasm and reserve food. After fertilization the fusion nucleus thus finds itself rich in the potentiality of reproduction, but poor in the materials necessary to make this reproduction effective. The sperms are non-motile and not particularly numerous, so that only a small fraction of the eggs are ever fertilized. There are present in the thallus other cells larger than the eggs and rich in protoplasm and in food materials corresponding to the yolk of the animal egg. These cells, known to the botanist as auxiliaries, lie at some distance, perhaps several millimeters, from the egg.

In this situation the egg sends out a filament, the oöblastema. The fusion nucleus moves to the tip of this filament and there divides once by a modified mitosis. The oöblastema creeps forward in the thallus without demonstrable chemotropic direction, touching cells here and there as it pushes its way through the soft gelatinous chinks between the body cells until it touches, as if by accident, an auxiliary cell. The tip then bends toward the auxiliary and fuses with it, and the two fusion or diploid nuclei pass into the rich cytoplasm found there. The haploid nuclei already present disintegrate, leaving the diploid nuclei from the egg plus the cytoplasm of the auxiliary to replace the original egg in producing the new individuals.

The stimulus of the entry of the diploid nuclei has a remarkable effect on the activity of the auxiliary. It proceeds at once to divide repeatedly and forms finally a spherical mass of spores, numbering several hundred and each of large size and provided with an abundance of stored food. This activity of the auxiliary is associated with a hardness unusual in such delicate cells as characterize the red algae. In one specimen, laid open and placed in sea water on the slide just as the oöblastema filament was making contact with the auxiliary, it was noted that the vegetative cells of the body, including those

auxiliaries which had not received diploid nuclei, were all dead, their chloroplasts laked like blood corpuscles, within 30 minutes. The auxiliary, however, remained healthy and continued to divide under most trying conditions for seven hours.

The stimulus here is the presence of the fusion nucleus, the problem is to manipulate it in such a way as to insure effective reproduction. Transfer of nuclei for considerable distances in plants is seen in pollen tubes, but the red algae are unique in moving the nucleus from the egg itself to an entirely different cell some distance away. This manipulation of the egg nucleus plus cooperation of the auxiliary greatly increases the production of new individuals following a successful act of fertilization and thus compensates for the frequent failure of fertilization to take place. The number of egg cells is much greater than that of auxiliaries so that the wastage in the frequent failure of fertilization is minimized.

Of intra-organismal stimuli I may mention the unexpected behavior of cambium cells as shown by Bailey.²

In the white pine the dimensions of these cells have been given as 4,000 by 42 by 12 micra. Division planes along the short axis would be confidently expected by the cytologist. Actually division is in the plane least to be considered possible, and the long thin sheet-like cell is split into two still thinner sheets of the same length and breadth. In this unusual behavior, which in its defiance of physical laws is almost like making water run up hill, the cambium cell, of course, is reacting to some stimulus the nature of which is entirely obscure. The result, however, is a happy one, producing the very long cells needed in the conducting system of the stem.

The formation of the spicule in certain fresh-water sponges³ is another example of cooperative action by which cells attain what may fairly be called an objective of survival value to the sponge. A single cell, the spicule-forming cell or primary silicoblast, elongates and lays

² *Am. Jour. Bot.*, Vol. 7, 1920.

³ Schröder, *Zeitsch. f. Morphologie u. Oekologie der Tiere*, Vol. 31, 1936.

down an axial filament in the cytoplasm, the cell later dying. This filament is coated with silicic acid. After the silica content of the spicule-forming cell is used up in this way, amebocytes with large vesicular nuclei move up. These cells contain large clear vacuoles which come to show under dark-field illumination the same glassy appearance as the silica of the spicule itself. The amebocytes are secondary silicoblasts. One moves to a spicule cell, joins it and then pinches off the vacuole containing silica gel, along with some cytoplasm. The silica vacuole is left on the young spicule and gradually spreads over it, increasing its thickness.

When the spicule reaches a certain length, a second type of amebocyte, without a vacuole and with a small granular nucleus, moves to the spicule and joins it. As many as eight may become attached to the spicule. They transport it to its place of functioning in the body of the sponge.

Here we have three different kinds of cells cooperating to form and transport the spicule. One lays it down, another brings up and delivers silica to it, the third transports it to its functional destination.

Even more remarkable is the response of certain cells of the flatworm *Microstomum* to the nematocysts of *Hydra*.⁴

There are four types of nematocysts in the species of *Hydra* studied. Two of these, the penetrants and streptolines, when discharged penetrate the body of the victim or enemy. Upon discharge, both thread and bulb are shot out from the *Hydra*'s body. The other two discharge only their threads, leaving the bulbs still embedded in *Hydra*'s ectoderm. In one of them, the stereoline, the end of the thread is adhesive and may become affixed to the surface of the victim. In the other, the volvent, the thread acts as a lasso, coiling around a hair or other minute projection of the victim's body. Obviously, the penetrants and streptolines are offensive-defensive, while the

⁴ Kepner, Gregory and Porter, in *Zool. Anzeiger*, Vol. 121, 1938.

stereolines and volvents are functional in capturing and holding prey until it is dragged into the mouth.

Microstomum kept for 50 generations without access to *Hydra* will eagerly ingest tissue of the polyp when it is presented. The fate of the ingested nematocysts has been carefully followed. All four types are first taken in by the non-glandular cells of the enteric epithelium or endoderm, where they lie in large vacuoles. These pass through the basement membrane of the endoderm and come to lie in the intercellular spaces of the mesodermal parenchyma. Here the stereolines and volvents are digested, but the penetrants and streptolines are engulfed by attending parenchyma cells and transported by them outward to the epidermis. The attending cell is then encapsulated by a second parenchyma cell. It loses much of its chromaticity during these movements and shows an irregular, shrunken nucleus, while the capsule cell stains normally. When the transporting cell comes into contact with the epidermis of *Microstomum*, the latter becomes deeply pitted both internally and externally, but does not rupture. The nematocyst is oriented within the transporting cell in such a manner that it points outward just as it did in the *Hydra* from which it came. It is also functional just as in *Hydra* and serves as an offensive-defensive weapon. It should be noted that the stereolines and volvents, which could be of no use to *Microstomum*, are digested within the parenchyma.

In this case we see a thoroughgoing cooperation among the cells of *Microstomum* in the manipulation of nematocysts of *Hydra*. The endoderm cell takes them in and passes them into the mesodermal parenchyma. Here the cells distinguish between the useful and non-useful kinds, digesting the latter and transporting the former to the point of functioning and orienting them there. Then the cells of the epidermis respond by producing a pit through whose thin membrane the nematocyst discharges its stinging thread upon suitable stimulation.

Considering the various examples of the striking complexity of the beautifully ordered reactions of cells, all

leading to the development of survival values to the individuals, though the cells themselves may perish, one sees an analogy to the social insects. In a hive of bees, each individual has some task and does it with the end result that the swarm survives. It has been said that the "spirit of the hive" directs and controls the activities of the individual, heedless of individual welfare, but most careful of survival of the species. Certainly the behavior pattern of the swarm and of the cells is very similar. In the case of the cells, perhaps some organizer or group of organizers may be found to be responsible for the complex set of cooperative reactions. That some force is directing the physical and chemical factors involved in the successive stimulations of cells to do some particular thing at a certain moment seems an inescapable conclusion. It is just as likely mathematically that *Microstomum* without directive control can manipulate *Hydra* nematocysts successfully and safely as it would be for a sonnet to emerge from a random serial selection of a certain number of letters from a jumbled pile of alphabetical characters.

In the third category the stimuli are extra-organismal, and here we have the best opportunity for their analysis. The most striking examples are found in insect galls of plants where, in response to stimuli apparently chemical in nature, most elaborate and beautifully effective structures are produced by various plant tissues. The "spirit of the hive" idea breaks down in this case, since the response by no means leads to the survival of the plant. Directive control of the cellular response comes from the insect larva, whose secretions must furnish the orderly succession of stimuli that bring about reactions similar in every respect to the normal life process itself. Growth, differentiation and form all emerge as a result of the larval activity. Spatio-temporal relations are established and maintained in the plant organ involved of the same nature as those seen in normal differentiation. I know of no material, animal or plant, that offers so likely a field of

attack on the most fundamental of biological problems. If some young physiologist, provided with the armamentarium afforded by the latest advances in biochemistry and biophysics, should undertake the analysis of the control exercised by the insect larva on plant differentiation, he might make a nearer approach to the mystery of life than has hitherto been possible.

One need not suppose, however, that such an investigation, be it ever so successful, will settle the perennial argument between such wholehearted protagonists as those distinguished physiologists, J. S. Haldane, on the one side, and the late Jacques Loeb, on the other. We would probably agree that there is a residuum for which no physicochemical description has yet been found. The question of whether it can ever be found is a philosophical rather than a biological one. The biologist can well stand aside from such a metaphysical problem and should certainly keep an open mind and not accept either the crass oversimplifications of some mechanists or the retreat from reality of some vitalists.

In the last 300 years this residuum has steadily been whittled down. Whatever a biologist believes in his heart, he must assume in his research that there is in this residuum, this "alogical core of the universe," no citadel that may not be taken. As Needham⁵ says, "the minimization of the alogical core of the universe, then, is the proper pursuit of science."

To assume, however, as a basis of research that there is no unknowable is one thing, but it is quite a different thing to deny in daily life the reality of "the substance of things hoped for, the evidence of things not seen." The scientist has developed his special technique by a deliberate abstraction. The scientific method, the most wonderful tool ever produced by the mind of man, is based on this abstraction, by which we tacitly agree to assume that everything is spatial and therefore within the possible reach of the senses. It is not necessary for me to say to

⁵ "Order and Life," p. 13, Yale University Press, 1936.

this audience that by use of this method man's comprehension of and control over nature has constituted a veritable grand slam, the only one perhaps to which he can lay claim throughout the long course of his struggle with nature. In our own memory, among the many far-reaching discoveries and applications of science to revolutionize our lives, it is said that the life expectancy has been raised by 21 years. Nevertheless, I get the impression from talking with many young people that the worthwhileness of life has sensibly diminished.

One of these young people might say: "You have shown that man is a natural object, subject to the same physiological laws as other animal organisms and part of a comprehensible natural order. You have shown that the actions of such animal mechanisms are nothing but tropistic responses, or at best conditioned reflexes; that the way of a dog with a bone, or with his master, or with other dogs, is of the same order as the way of a magnet with steel, or the behavior of an acid in the presence of a base. You have shown how inexorable tropisms (and taxies) are. You have stressed the analogies of living things. The dog howls when he is kicked for exactly the same reason that a bell rings when the rope is pulled. Therefore we are not responsible for what we do, since the immediate cause of all our actions is the tug of circumstance. We are at the mercy of every wind that blows, winds over which we have no control and about which we know nothing. There is no such thing as responsibility in an individual ridden by fate in all he does. It is not my fault when things go wrong."

Ladies and gentlemen, the relief rolls are based on that philosophy—the bodily bankruptcy that we see around us is a mirror image of a spiritual bankruptcy that is merely the reverse of a scientific philosophy whose obverse is bright and shining with hope for a better world.

Science has swept the field, but in doing so has extended to the daily life of people its own peculiar abstraction. Moral values belong to the unseen and probably the unsee-

able. To ignore the existence of values by leaving them outside the field of science has justified itself by many exciting results in the laboratory that seem to promise an ever more extensive control over the forces of nature and a clearer and wider understanding of the world and the universe.

There is, however, another side to the picture when the non-scientific public, somewhat dazzled by the solid results of research that so easily become the "miracles of science," begins to apply to everyday living the formula that has been so fruitful in research. Is it not true that the political, social and economic mess in which the world now finds itself may be traced to the same great abstraction that has meant so much to scientific research? It is only a very short step from saying, "The only reality is what we can see," to regarding a treaty as a scrap of paper having no values beyond the visible ones. Adolph Hitler, when he made his Munich promises in 1938, was thinking along the same lines as Bethmann-Hollweg in 1914 when he made his famous remark about the treaty guaranteeing Belgium against aggression. Both merely transferred to the field of *Weltpolitik* the technique useful in the field of science. That such transfer is dangerous is shown by the most casual glance at events in Russia, Poland and Finland in the last few months.

The repudiation of obligations when such repudiation becomes convenient has become a veritable disease, epidemic among the nations of the world. Even the most upright of the democracies has shown the symptoms. Our own country once obligated itself to pay certain bonds in gold or its equivalent. It became inconvenient to do so. By the science of economics it was concluded that ponderables would be conserved by a repudiation of the pledge given. Many suspect, however, that imponderables of far greater value were sacrificed than were compensated for by the money saved.

Napoleon is said to have stated that God is on the side having the heaviest artillery, but one of the most realistic

of nineteenth century statesmen, Bismarck, is quoted as saying that wars are won by imponderables. Now imponderables are not found in laboratories, but would it not be a service to clear thinking if we admit their existence elsewhere?

There is no indication that the age-old controversy will ever be settled. Perhaps by its very nature there is no solution, but most people lean to one side or the other. The attitude of the scientist may well be that of trial and error. We are forced back on the pragmatic. The best results seem to be attained by ignoring values in the laboratory and by recognizing them in that part of the world lying outside the ivory towers.

I am grateful to the society for this opportunity to appear before you. I am reminded of Sam McKeithan. A party of gentlemen went to fish on the Waccamaw River down in North Carolina. They were sitting around the fire after supper and telling tall tales of deep-sea fishing and the whoppers they had caught in the Gulf Stream. The guide sitting in the corner listened quietly for a while, then shifted his quid and spat, and remarked, "That reminds me of Sam McKeithan. There was a big fish in Lake Waccamaw, and some of us thought we'd catch it. We got us a plow line and got the blacksmith to hammer us out a hook. We took a plowshare for a sinker and a gum log for a cork and half a hog for bait. Well, we hadn't been fishing ten minutes when we got a strike. We all pulled, and we drug up to the bank the biggest fish you ever saw. 'Laws a mercy, boys,' says I, 'ain't that a pair of boots sticking out of his mouth?' So we caught hold of the boots, and out came Sam McKeithan. He wiped the mud and slime off his face, and he says to us, 'Gentlemen, I'm much obleeged.' "

Well, I am very much "obleeged," and I wish you all a very happy New Year.

LOCAL AND GENERALIZED DEFENSE REACTIONS IN PLANTS AND ANIMALS

LOCAL REACTIONS IN PLANTS¹

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As in many subjects, there are a number of different sides to the problem of local responses of plants to parasitic attack, and the problem can be viewed from many different angles. So far, however, the greater emphasis has been laid on this problem by the plant pathologists, whereas the field has been sadly neglected by the plant physiologists. This means that these local reactions have been considered mainly from the standpoint of the parasite, whether or not the latter was facilitated or hampered by the response of the host plant. Of course, this side of the problem is most important to the pathologist and the disease-control officer who have to consider in the first place the spread of the disease and must devise methods of checking its growth. The plant physiologist, on the other hand, is more likely to ask how the host happens to react in such a specific way to the parasite. In his case, the parasite is not the problem, but only a means of studying certain reactions of the host. Therefore, what the pathologist takes more or less for granted, namely, the host's response to the parasite—is the specific problem of the physiologist. The latter is not much concerned with the question of the extent to which the reactions of the host plant control the parasitic attack, whether they are beneficial, whether they are useful adaptations, etc.

The limitations which are imposed on me are mainly limitations due to my own specialization as a plant physiologist and a time limit which prevents exhaustive discussion of the problem. Therefore we will take up a few special topics which seem to me of most interest and which

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give a rough survey of local reactions to parasitic attack in plants from the standpoint of the host, taking its growth reactions as a starting-point.

When consulting a text-book of plant pathology it will be evident that reactions to parasites run the whole gamut from death to stimulated development, and there are very few reactions which our fancy could imagine, that are not realized.

Let us start with the simplest case, namely, death, in which the parasite before or after penetrating the tissues kills the host cells. Let us distinguish in this case between obligatory parasites requiring living host cells—such as rusts, and the more saprophytic parasites which live only on the contents of dead cells. The latter parasites are least specialized and the local reactions of their hosts are simplest. In general, they cause small or larger injuries in the form of necrotic spots in mature tissues, or complete collapse of immature tissues in growing organs. Most of these parasites are unable to penetrate the cell walls and grow only between the cells in intercellular spaces or by dissolving the pectins of the middle lamella. To collect the food inside the cells of the host their semi-permeability has to be destroyed, and this is achieved by killing the cells. In many cases these parasites excrete specific toxins which diffuse in the host tissue, killing all cells as soon as their concentration has reached a certain limit. As soon as death of the host cell has occurred, their sugars and other diffusible food material will leach out and become available to the parasite which is waiting outside the cell wall of these cells. This simple reaction also explains why so many saprophytes follow in the wake of these less specialized parasites, for, when once a parasite has killed the host cells, any saprophyte can live on the freed cell contents. If the host cells are in excellent health the toxins are less effective and the plant may not be attacked. From a physiologist's standpoint, these fungi are of little interest. It is the crudest form of parasitism. If we compare these relations with our own social structure we can com-

pare this case with highway robbery. Just stab the victim and, when his resistance has been nullified by death, take whatever you want from him.

There is a somewhat more interesting angle to this problem when the parasite does not progress too rapidly inside the host tissue. Then the host may be able to form a layer of wound cork around the attacked region. In this case we have to do with an interesting physiological reaction which is already understood to some extent. Haberlandt had shown that wounded cells liberate diffusible substances which stimulate cell division. These he called wound hormones. In conjunction with lepto-hormones coming from the phloem they are responsible for cell divisions and the formation of, for instance, the cork layer at the cut surface of a potato tuber. One of these substances, traumatic acid, has now been isolated and synthesized and, presumably, the isolation of others will follow in time. With this knowledge at hand we can now form the following picture of the cork layer formed around the infected areas. Let us consider a parasite which has entered the plant, killing cells and liberating all cell contents, including wound hormones. The food is taken up by the parasite and the diffusing traumatic acid will stimulate the surrounding cells to divide. If the rate of killing by the parasite exceeds the rate at which traumatic acid can induce a protective cork layer, the infection will spread, but if, on the other hand, the traumatism has acted before the diffusing toxins have killed the cells a protective layer will be formed around the infected area. A very typical result of such cork-layer formation is the shot-hole disease in cherry leaves. The killed tissue around the parasite (*Coccomyces prunophorae*) has been surrounded by a cork layer and has been severed along this layer leaving small, round holes in the leaf. Also, sulfur dioxide and other gas injuries become surrounded by such cork layers. In the latter case it is easy to see how the cork layer could form since no spread of toxins prevents the cork formation.

In this case a very apt comparison can be made with

cities of the Middle Ages. Before they were surrounded by a protective earth-and-stone layer and gates they were periodically ransacked. But if they were not completely destroyed, they could build protective layers and the infestation was checked.

In the case of obligatory parasites, such as rusts, the conditions are practically reversed. The parasite can only live at the expense of the living cells of the host. Its hyphae penetrate the cell walls, and then their protoplasm seems to mix with that of the host. The more susceptible the host, the fewer necrotic cells. But when the host is resistant, it means that the rust, when it penetrates cells, kills them, which necrosis may spread so that typical discolored areas around each infection appear. The rust can not live on dead cells and, therefore, dies if the host cells are dead. Here the local reaction of the plant, leading to resistance, is of the simplest type. The parasite is of the beggar type. It can exist only as long as the host is sympathetic, but it has to succumb when the attitude of the prospective donor is unfavorable. It is a true symbiosis.

There are a number of such obligatory parasites which so far have resisted all attempts to grow them in culture solutions. Apparently they receive from the host plant substances which are destroyed upon the death of the host itself. In general these parasites are the most extreme in their specialization so that they are able to attack only one, or few species, and within the species again only certain varieties. In many cases they also have a most remarkable succession of fruiting stages which alternate on very different hosts. This specialization is a remarkable physiological problem and might be due to and enable the analysis of protoplasmic specificity, but so far plant physiologists have not followed up this problem. Perhaps it is still too complex to solve with our present knowledge and methods.

Having discussed the reactions leading to death of the host cells, we now have to consider those cases in which

the parasite modifies the development of growth rate or form of the host.

Reactions to simple wounds inflicted by browsing cattle or other herbivorous animals are quite interesting, but hardly fit into this discussion. Very seldom, if ever, will the response be different from those following experiments in which parts of the plant have been removed by cutting. Also, reactions to man-inflicted injuries, such as tree surgery, lawn mowing² and pruning, would come under this heading.³

Of great interest are all changes in form or growth rate or development in general, which are induced by parasites. In many cases structures develop which under normal conditions are not present in the plant. We only have to think of corn smut, nematode galls on roots, insect galls, bark canker, crown gall, to realize what remarkable effects parasitism may have on the host. Naturally, one asks whether the parasite is able to bring about completely new potentialities, which are not present as such in the host. The answer to this question can be given only after a much closer analysis of the facts. To begin with let us take the case of crown gall, a parasitic outgrowth common on many plants, which has been analyzed in considerable detail.

After a bacterium, *Phytomonas tumefaciens*, has entered the plant, usually through some wound, a tumor-like enlargement develops at the place of infection. The first symptoms may appear five days after infection but mostly a longer period elapses. The growth of the tumor may continue for a very long time, until large swellings have developed. The rate of growth of the swelling depends upon the condition of the host plant; when the latter is in a resting period, little or no growth of the crown gall oc-

² It might be pointed out, however, that some of these reactions are distinctly advantageous. A meadow which is regularly pastured or mowed has a completely different vegetation than when left untouched. Only those plants which can easily regenerate buds from roots, or in which the lower axillary buds can grow out after cutting the tops off, or which form prostrate shoots will survive.

³ This all comes under the general heading of "survival of the fittest."

curs. A rapidly growing gall will inhibit the development of other galls on the same plant. This all indicates that the gall which is produced is a cooperative undertaking in which the host is not passive. The host not only furnishes all cells and food materials necessary for growth of the gall, but also its condition is reflected in gall growth. The tissue which is formed is generally rather undifferentiated callus tissue, which especially on woody hosts later may become woody through differentiation of wound tracheids. Sometimes roots or shoots will develop on the surface of these galls, giving rise to so-called "hairy root." All these reactions may occur on plants after simple injury, without bacterial infection. The callus formed on poplar cuttings, *e.g.*, resembles crown gall.

While all these facts already indicate that the effects of the crown gall parasite are not due to highly specialized activities, the relative simplicity of the parasite-host relations in crown gall is clearly demonstrated by the fact that on many plants application of high concentrations of certain growth-promoting substances will induce swellings which closely resemble crown gall infections. Although it has been demonstrated also that *Phytomonas tumefaciens* can form growth-promoting substances *in vitro*, the available evidence rather points to the conclusion that the parasite does not induce gall formation on the host by simply producing growth-promoting substances. In the first place, the auxin concentration produced by the bacterium is so low that if applied by itself it would not cause any effect. In the second place, non-pathogenic bacteria may form the same amounts of auxin. And in the third place, there are many plants which do not respond to auxin application but which produce large crown galls with *Phytomonas*.

At first sight one is inclined to consider the considerable outgrowth due to *Phytomonas* as a useful adaptation, and the ability of the latter to induce such excessive growth of the host as purposeful and advantageous. But such a general consideration meets with difficulties: the shoots and

roots developing from the crown gall are apparently not infected. Therefore, we have to admit that at least some of the responses following bacterial infection are accidental and not useful, and are a natural sequence of the reactions started by the parasite. This means that many reactions induced by the parasite are accidental rather than purposeful. And when we consider the gall as a whole, doubt arises as to the usefulness of the large outgrowth. Certainly the bacteria represent only a very minor fraction of the total bulk of tissue formed, and even if no major swelling occurred, would have sufficient food to draw upon from the host.

Since every plant pathologist knows how difficult it is to sterilize completely many living plant tissues, even when outside infection of healthy organs is practically excluded, we come to the conclusion that in many apparently healthy plants bacteria live, which, although drawing upon the food supply of their host, do not induce abnormal growth. Therefore it seems not unlikely that the reaction induced by the crown gall parasite is only, let us say, an accident, not essential for the proper growth and development of *Phytomonas*, but based on a constellation of accidental factors, which induces growth of the host, just as application of indole acetic acid causes cell growth.

Other abnormal growth reactions are caused by fungi which induce witches'-brooms. Under the influence of the parasite the normal inhibition of lateral axillary buds by the apical bud is abolished, and most laterals grow out. The mechanism of the formation of witches'-brooms has not been determined as yet, but it seems likely that it can be explained completely in the light of our present knowledge of bud inhibition as an auxin phenomenon.

Some remarkable reactions are caused by those parasites, which make organs develop where otherwise there would have been none. A typical case is that of *Ustilago violacea*, which causes the formation of anthers in the female flowers of *Melandrium rubrum*, a strictly dioecious plant. In this case, the parasite only brings out a poten-

tiality of the host species, which otherwise in the particular individual (female plant) does not express itself.

We will conclude this survey with a description of some malformations, etc., caused by animals. These may be due either to decreased or increased growth. A very simple malformation is the crinkling of leaves produced by early infection with aphids. These animals penetrate with their mouth parts some of the sieve tubes. If they attack young plant parts, then all growth due to auxins—that is, cell elongation of stem, petiole and leaf veins—is decreased. Mesophyll growth continues at a more normal rate, and this causes a bulging of the intercostal areas of the leaves, giving rise to the formation of pockets in which the aphids are well protected. The effect of such aphid infection can be explained satisfactorily by assuming auxin destruction in the phloem. In other cases, excessive elongation of cells or organs occurs, such as erineum (which sometimes can be induced by application of indole acetic acid), which can be attributed to increased auxin content of the reacting tissues.

But the most complex structures are the galls formed by gall midges and gall wasps. These galls are structures of determinate growth, mostly of very uniform size, and have forms which differ very much from anything normally produced by the plant. They are built out of tissues of the normal plant, but these have been organized in a very different and definite pattern.

The stimulus for this abnormal organization almost invariably comes from the egg or young developing larva of the gall insect, and except in the case of the *Pontania* or *Capreae* gall, not from the egg-laying insect. In some cases the larvae even crawl to the place where the gall is going to develop, so that the deposition of the egg has nothing to do with the gall. This is also demonstrated by the fact that if the gall wasp fails to lay eggs in the incisions it has made, or if the eggs are removed soon after laying, no gall develops. This all means, then, that the galls are

local responses to the activity of the larvae of the gall insects.

The complexity of the structures induced by the gall insects is often astounding. The central part of the gall with the insect in it may become detached after it is full grown. Then the insect will be released from this box through opening of a pre-formed lid. Often very special structures develop enabling the full-grown insect to escape from its perfectly closed larval chamber. The inside of this larval chamber is often lined with cells very rich in proteins.

Only in the case of the *Pontania* gall on willow leaves, Beyerinck found that it would develop even if no egg was deposited. But in this case the insect, or tenthredonid, injects a small amount of material from a special gland into the incision made with its ovipositor, and this by itself is sufficient to make the gall develop. Here we have the first published concrete example of a substance causing differentiation and growth, and Beyerinck named it a "growth enzyme."

In general, we can say that the study of those local reactions which we call galls will yield the most interesting material for analysis of developmental processes. With the example of the insect before our eyes, we know that influencing the normal growth of a plant is possible and we should not rest until we have imitated the gall wasps in their effects on plants.⁴

It does not seem to lie within the range of my subject to discuss certain cases of immunity due to preformed anatomical or physiological characteristics of the host plant, such as disease-escaping immunity, pH of cell sap which excludes chemotropic attraction of parasite, extra heavy cuticle, thorniness of branches which prevents birds depos-

⁴ In the previous discussion we have met parasites of very different types, such as robbers and beggars. To round out our comparison, I propose to nominate the gall parasites as gold-diggers. For they are able to induce the host to excesses far beyond anything they provide for their own offspring and also far beyond the needs of the parasite. And, as in human society, certain plants, such as oaks, are notorious as suckers.

iting seeds of parasitic Loranthaceae, etc. Those are all defense mechanisms, but are not local reactions. They are of importance for the plant pathologist, but lie outside the field of the physiologist.

In the preceding discussion we have seen that there is a very wide variety of local responses of plants to parasitic attack, ranging from death of the cell to greatly increased growth and even the formation of apparently new forms. But in all cases the new formations are definitely within the potential range of development of the host, and in some of the simpler cases these new forms have been induced by chemically known substances. What the parasite does is to bring out potentialities, rearrange tissues, and develop new growth patterns. Here lies a rich field for cooperation between pathologist and physiologist.

GENERALIZED DEFENSE REACTIONS IN PLANTS¹

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INTRODUCTION

SOME plants are naturally immune or resistant to diseases that attack other closely related plants. This natural immunity may be due to either morphological or physiological conditions that prevent entrance and subsequent growth of the parasite in the plant body. Many parasites are dependent upon the presence of natural or artificial openings in order to cause infection. When such openings are absent, the host is immune. On the other hand, the parasite may readily gain entrance to host cells but find conditions there unsuited to its further development. For example, the resistance of red and yellow onions to the onion smudge organism, *Colletotrichum circinans* (Berk.) Vog., was shown (Walker, 1923; Walker, Link and Angell, 1929) to be due to the presence of protocatechuic acid in the outer scales of these onions.

Although natural immunity in plants has been recognized for a long time, it is only in recent years that good evidence for the existence of acquired immunity has been obtained. In a symposium on defense reactions in plants and animals, it is appropriate to review this evidence. The review will be concerned only with virus diseases, since there is some question whether acquired immunity from bacterial and fungous diseases has been demonstrated in plants.

THE MEANING OF ACQUIRED IMMUNITY

At the outset, it is well to state clearly what is meant by the term "acquired immunity." It is the specific *resis-*

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tance an individual organism acquires to a disease. The fact that the resistance is specific differentiates it from changes in susceptibility induced by nutrition or aging of the host and by such environmental factors as would be apt to alter resistance not only to the disease in question but also to various other unrelated diseases. Acquired immunity may be either active or passive: It is active if induced by infection with a parasitic agent, passive if caused by injection with immune serum or antibodies obtained from other individuals. Passive immunity has not been demonstrated to occur in plant diseases.

It is convenient to classify active immunity into three types, the chronic-disease type, the carrier type and the sterile type. In the chronic-disease type, neither the immunizing disease nor the causal agent disappears, but the individual is solidly immune from closely related diseases. In the carrier type, obvious manifestations of the disease disappear, but the causal agent persists. However, the individual may be protected from a second attack of the disease even when subjected to massive reinoculations with the infectious agent. In the sterile type, the immunity may be transitory or permanent and readily or with difficulty broken down by reinoculation; both obvious signs of disease and the inciting agent disappear or at least can not be detected by ordinary methods. The sterile type has not been demonstrated for plant-virus diseases. It must be recognized that there is no sharp line of division among the three types of acquired immunity. The extent to which the pathogenic agent disappears determines whether the immunity is of the sterile or carrier type, and the extent to which symptoms disappear determines whether it is of the carrier- or chronic-disease type.

For the purpose of the present discussion it will be more convenient to divide acquired immunity into the immunity which follows recovery from disease, and cross immunity, in which one strain of a virus protects a plant from infection by closely related strains. Immunity following recovery from disease is usually of the carrier type while cross immunity is more commonly of the chronic-disease type.

IMMUNITY FOLLOWING RECOVERY FROM DISEASE

There are at least six different virus diseases from which plants recover and acquire immunity. These are tobacco-ringspot (Price, 1932), tomato-ringspot² (Price, 1936), tobacco streak (Johnson, 1936, 1937), Bergerac-ringspot of tobacco (Smith, 1937), sugar-beet curly top in certain races of tomato and in tobacco (Lesley and Wallace, 1938; Wallace, 1939) and potato yellow dwarf (Black, 1937, 1938). Of the six diseases, tobacco-ringspot has been the most extensively studied.

The virus of tobacco-ringspot is readily transmitted by rubbing juice from a diseased plant over the leaves of susceptible plants. Tobacco (*Nicotiana tabacum* L.) plants inoculated in this manner develop a characteristic type of lesion on inoculated leaves in about three days and systemic lesions of a similar type in three or four more days. The lesion consists of concentric necrotic rings alternating with normal green tissue. In from twelve to thirty-five days after inoculation, diseased tobacco plants begin to recover. Recovery consists in the production of new symptom-free growth and eventual loss of old affected leaves. The first indication of recovery is the development of leaves with necrotic patterns resembling the outline of an oak leaf. The basal portions of such leaves are healthy in appearance, while the apical portions frequently show zonate necrotic lesions. As new leaves are produced, the lesions and oak-leaf patterns are restricted to progressively smaller areas at the leaf tips. Finally, such symptoms fail to appear altogether. Subsequently produced leaves resemble those of healthy plants but are slightly darker in color, somewhat thicker and of a more leathery texture. Recovered plants appear to grow somewhat less rapidly than healthy plants. Wingard (1928) transmitted tobacco-ringspot to plants of thirty-eight genera in seventeen different families and reported that recovery from the disease occurred in practically all the plants tested. Re-

² This disease was previously referred to as tobacco-ringspot No. 2. The writer here uses the name proposed by Holmes (1939).

covery from tobacco-ringspot occurs under a wide range of environmental conditions (Price, 1932) and is different from the masking of symptoms (Johnson, 1921; Goss and Peltier, 1925; Bennett, 1927) of other virus diseases caused by exposure to special environmental conditions or by aging of the host plants.

Plants that have recovered from ringspot are solidly immune from a second attack of the disease (Wingard, 1928; Henderson and Wingard, 1931; Price, 1932). Plants grown under favorable environmental conditions from cuttings of recovered tobacco plants have been repeatedly inoculated with ringspot virus without the production of symptoms, whereas plants grown from cuttings of healthy plants and treated in exactly the same manner have invariably come down with the disease (Price, 1932).

By definition, acquired immunity is specific. Acquired immunity from tobacco-ringspot meets this criterion. Tobacco plants that have recovered from ringspot are susceptible to infection with tobacco-mosaic, cucumber-mosaic, potato-veinbanding, potato-ringspot, tomato spotted-wilt, tobacco-etch, tomato-ringspot, tobacco-streak, and Bergerac-ringspot viruses and, no doubt, to all other tobacco viruses that are not closely related to tobacco-ringspot virus (Price, 1936; Johnson, 1937; Smith, 1937). They are immune from or highly resistant to infection by closely related viruses such as those causing Valteau's (1932) green and yellow ringspot diseases.

That acquired immunity from tobacco-ringspot is of the carrier type is readily demonstrated by testing for the presence of virus in recovered plants (Wingard, 1928; Henderson and Wingard, 1931; Price, 1932). Indeed, the presence of virus in such plants led Valteau (1935, 1935a) to conclude that tobacco does not actually recover from ringspot. He maintained that symptoms are produced only when the growing point of the plant has not been completely invaded by virus, that leaves developed subsequent to invasion are parasitized although patternless, and that inoculation of patternless leaves is without effect since they

are already parasitized to the limit. Actually, however, it has since been demonstrated that virus does not multiply to nearly the same extent in recovered plants as in recently infected plants grown under the same conditions (Price, 1936; Stanley, 1939). The recovered portions of affected plants contain, on the average, only from 10 to 20 per cent. as much virus as the diseased portions of the same plant. Even in the same leaf, a partly recovered one, the diseased portion was found to contain considerably more virus than the healthy appearing portion. Furthermore, the virus concentration of recovered leaves is not increased by inoculating them heavily with ringspot virus.

Acquired immunity in tomato-ringspot, Bergerac-ringspot and tobacco-streak diseases is also of the carrier type and in other respects is similar to that observed in tobacco-ringspot (Price, 1936; Johnson, 1936, 1937; Smith, 1937). In each of these diseases, recovery is characterized by the appearance of oak-leaf patterns followed by production of leaves that are symptomless or nearly so. Recovered plants carry virus and are refractory to reinfection.

Recovery of tobacco and tomato from the curly-top disease (Lesley and Wallace, 1938; Wallace, 1939) may take place either by growth of healthy-appearing shoots from axillary buds or by production of progressively less severely diseased leaves from the terminal. In either case, the new growth shows a non-sterile immunity. In tomato, the immunity is not of so high an order as in the ringspot and streak diseases, since infected plants do not always recover and since those that do recover occasionally show a relapse and may, in a few instances, develop severe symptoms when reinoculated (Lesley and Wallace, 1938).

Recovery of potato plants from yellow dwarf likewise occurs by growth from axillary buds; such new growth shows chronic symptoms that persist throughout life. However, plants that have recovered from yellow dwarf in this manner may be grown through at least 5 vegetative generations without again developing acute symptoms of the disease (Black, 1937).

THE CROSS-IMMUNITY REACTION

Cross immunity in plant virus diseases may be either of the carrier or chronic-disease type. The protecting virus may be so mild as to produce no visible symptoms whatever or so severe as to cause marked distortion and chlorosis. In either case the plant cells are solidly immune from other closely related viruses, although fully susceptible to non-related viruses.

Thung (1931) was the first to study cross immunity in plant virus diseases. He isolated a yellow-mosaic virus from ordinary tobacco-mosaic virus and found that plants infected with the ordinary mosaic virus developed no change in symptoms when inoculated with the yellow-mosaic virus. Similarly, plants infected with the yellow-mosaic virus developed no change in symptoms when inoculated with the ordinary mosaic virus. Moreover, only one virus could be isolated from the doubly inoculated plants, the one first introduced. These results led Thung to conclude that only one virus could multiply extensively in any given cell.

Thung's results have been confirmed and extended by numerous investigators working with many different plant viruses. Perhaps the most striking example of cross immunity in plant virus diseases is that shown by *Nicotiana sylvestris* Spegaz. and Comes plants infected with certain strains of tobacco-mosaic virus. Kunkel (1934) found that mature leaves of *N. sylvestris* plants infected with mottling-type strains of tobacco-mosaic virus were immune from the necrotic-type aucuba-mosaic strain. That this immunity is specific was shown by the fact that leaves mottled by cucumber-mosaic virus or systemically infected with tobacco-ringspot virus were not, on the other hand, immune from the necrotic-type tobacco-mosaic virus.

Evidence that the cross-immunity reaction is strictly cellular was obtained in the following manner. When mature leaves of *Nicotiana sylvestris* plants, or portions of such leaves, were rubbed with mottling-type strains of tobacco-mosaic virus they became immune from the ne-

crotic-type strain of the virus within a period of two days. Those portions of the leaves that were not inoculated with the protecting virus did not become immune. This and similar evidence indicates that only those cells actually invaded by virus develop an immunity.

The cross-immunity reaction has been of considerable value in demonstrating virus relationships. As a case in point, acquired immunity of *Zinnia elegans* Jacq. from cucumber-mosaic virus is of interest. Mottled leaves of zinnia infected by any one of a number of different strains of cucumber-mosaic virus are immune from a necrotic-type strain (Price, 1935). Zinnia leaves mottled by other viruses are not immune from the necrotic-type cucumber-mosaic virus. It has been found that the viruses of Southern celery mosaic and lily mosaic both protect zinnia plants from the necrotic-type cucumber-mosaic virus (Price, 1935a, 1937; Ainsworth, 1938). This result led to the conclusion that both viruses should be classified in the cucumber-mosaic virus group, a conclusion supported by other lines of evidence.

THE NATURE OF THE DEFENSE MECHANISM

It seems clear from the evidence just summarized that plants can and do acquire immunity from certain virus diseases. The important question to be answered is: What is the nature of the defense mechanism involved in this immunity? Unfortunately, this question has not yet been answered. Nevertheless, it may be appropriate to discuss some of the theories that have been advanced to explain the acquired immunity.

Up to this point, I have spoken of acquired immunity in plant virus diseases as a generalized reaction. The fact of the matter is, however, that it appears to be generalized only because the viruses themselves are generalized; viruses usually become systemic a few days after their introduction into a plant. As mentioned previously, the available evidence indicates that the immunity is confined to those cells actually invaded by virus. Whether or not the immunity extends to nearby cells is, of course, a moot

question and not easily determined. It seems clear, however, that it does not extend further than, perhaps, the length of a few cells from tissues actually occupied by virus. It thus appears that acquired immunity in plant virus diseases is cellular in nature.

In the case of tobacco-ringspot, in which immunity follows recovery of plants from the disease, it has been postulated (Price, 1936) that the ringspot virus is able to reach its maximum concentration in, and to exert its maximum effect upon, only those cells that are mature or nearly so at the time of invasion. According to this view, invasion of embryonic cells is not accompanied by maximum increase in virus nor by severe injury to the cells; the embryonic cells become adapted to the virus and maintain an equilibrium with it. Hence, the plant recovers as soon as the embryonic cells become infected. Assuming this hypothesis to be correct, it still does not reveal the nature of the mechanism involved.

A more fundamental hypothesis for the nature of the defense mechanism is based on the assumption that certain protoplasmic constituents, such as, perhaps, specific amino acids, are essential for virus reproduction (Kunkel, 1934; Köhler, 1934; Bawden, 1934; Salaman, 1936). This being the case, one virus in a plant may exhaust the supply of material essential for reproduction of that particular virus and thus render the invaded cell immune from closely related viruses that require the same material. The cell would still be susceptible to unrelated viruses, since these viruses would not require exactly the same materials as the first virus. This hypothesis may serve to explain the cross-immunity reaction, but it does not explain the immunity which follows recovery of plants from tobacco-ringspot disease. In this case, the virus content of recovered tissue is considerably lower than that of diseased tissue, and it is to be inferred that either the essential material has not been used up or its production has been sharply curtailed.

The suggestion has been made (Thung, 1936) that virus-

infected plants, like animals, develop immunizing substances which neutralize or tend to neutralize the virus in the plant. In fact, Wallace (1939a) recently reported an experiment which he believes furnished evidence of production of antibody-like substances in Turkish tobacco plants infected with curly-top virus. When the virus is transmitted from recovered plants to healthy plants by means of the insect vector a typical severe disease is produced. But if the virus is transmitted by grafting scions from recovered plants onto healthy plants only mild symptoms usually result. The inference is that the grafting process allows transmission of immunizing substances as well as the virus, whereas the insect transmits only the virus. However, there are other explanations for production of only mild symptoms following grafting. It is possible, for example, that plants recovered from curly top contain both mild and severe strains of virus and that the grafting process transmits both strains while the insect transmits only the severe strain. Until such possible explanations have been eliminated it seems unwise to conclude that curly-top-virus antibodies are produced in plants.

When tobacco plants that have recovered from the ring-spot disease are grafted onto healthy plants, acute symptoms of the disease are produced in healthy plants. This indicates that antibodies, if they are indeed present in recovered plants, are not transmitted through the graft union (Price, 1932). Recently, experiments were made specifically to test for the presence of neutralizing antibodies in plants that had recovered from the ring-spot disease. On the assumption that antibodies might be present in juices of such plants, several treatments were resorted to in an attempt to separate the virus from the antibodies. Juice from recovered plants and juice from diseased plants were subjected to the same treatment. These juices were then added in various proportions to juice from diseased plants; the resulting mixture was allowed to stand for an interval of time and was then tested for virus concentration by inoculation of 40 leaves of the

cowpea (*Vigna sinensis* Endl. var. Black). The data obtained from these experiments are summarized in Fig. 1.

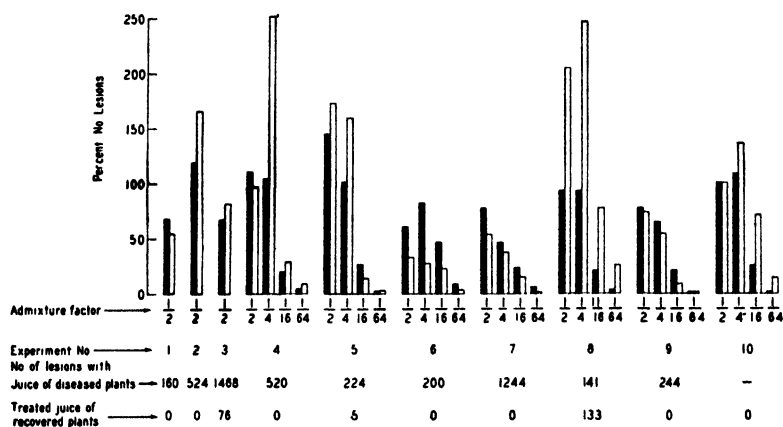


FIG. 1. Results of attempts to demonstrate neutralizing antibodies in plants that had recovered from tobacco-ringspot. Black columns represent relative numbers of lesions produced on sets of 40 cowpea leaves by diseased-plant juice diluted with various amounts of healthy-plant juice; white columns, by diseased-plant juice diluted with various amounts of recovered-plant juice that had been treated to remove virus. In each case the number of lesions is shown as percentage of the number produced by undiluted juice of diseased plants.

In experiments 1–6, juices of recovered and healthy plants were heated to 60 or 70° C. for 10 minutes before mixing them, in proportions up to 64 to 1, with juice of diseased plants; in experiments 7–9, the juices were filtered through a Berkefeld W filter candle before mixing; and in experiment 10, the virus-free supernatant from an ultracentrifuged sample of juice was used. The presence of antibodies in the treated juice of recovered plants would be expected to lower the infectivity of the virus to which it was added. Also, the reduction should be more pronounced as the proportion of recovered-plant juice was increased. Study of Fig. 1 shows that the addition of juice of recovered plants caused no reduction in lesions beyond that caused by addition of juice of healthy plants. Thus, no evidence was obtained that neutralizing antibodies were present in the recovered plants. In evaluating these data, however, it must be remembered that the

treatment used to destroy virus or otherwise remove it from recovered-plant extract may likewise have removed antibodies, if such were present.

It is clear that present theories are inadequate to account for acquired immunity from plant virus diseases. While later study may show that antibodies play a part in this immunity, it seems more likely that the defense mechanism is of an entirely different nature.

SUMMARY

To sum up briefly, the data accumulated during the past decade show conclusively that plants infected with certain virus diseases develop a defense reaction beyond the natural immunity inherent in the plant. There are some virus diseases from which plants recover and become refractory to reinfection. The immunity that ensues is of the carrier type, since the plant continues to harbor the virus. In many instances—in all instances so far studied—plant cells infected with one strain of a virus become immune from infection with a closely related strain. In this case, the immunity is usually of the chronic-disease type, since the plants lose neither the virus nor the symptoms produced by the virus. In both types of immunity, the nature of the defense mechanism is unknown. The nature of the mechanism is of importance not only with respect to plant virus diseases but also in the general field of immunity as a whole, for even in animal diseases the exact nature of the defense reaction is by no means solved. It is to be hoped that further studies on plant virus immunity will at least indicate the byways along which we need to go in pursuit of this elusive question.

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LOCAL AND GENERALIZED DEFENSE REACTIONS IN ANIMALS¹

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IN reply to the question "What do you understand by 'injury' and the mechanisms of defense against it?" the students of the various biological disciplines give distinctly different answers. Some of the defense reactions in plants have just been discussed by the previous speakers. The biologist studying protozoa would probably discuss their reactions to starvation and poisons and predatory animals. The pathologist would reply in terms of parasites, inflammation, immunity. And further diversities of content appear in the answers of physiologists, biochemists, psychiatrists and the students of social organization.

One may say in general terms that anything which tends to interfere seriously with the local or general equilibrium in an organism tends to injure it; that defense is the reaction of the organism to ward off an injury or prevent its spread; and that repair is the attempt of the organism to return toward the state before injury. But as soon as we try to delimit these terms more closely, we encounter the almost universal difficulty of making consistent definitions of biological concepts. This results primarily from the extremely closely graded series and their overlapping of biological forms and processes.

Link encountered a similar difficulty in attempting to distinguish health from pathic states: "Sooner or later, in the course of its life cycle, events may and usually do occur within an organism with more or less frequency during which its reactions to internal and external stimuli

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exceed the limits of easy tolerance or even of its absolute adaptive capacity." My discussion will be centered in large part on those processes which he encloses in the phrase "easy tolerance."

In addition, the analysis of defense reactions has been confused by the introduction of mystical and anthropomorphic concepts and of teleological interpretations, as well as by the refusal of many biologists to admit that adaptiveness, even though explainable in physical and chemical terms, is characteristic of living reactions.

Living organisms are the seat of a continuous interplay between a wide variety of stimuli and the reactions which they call forth. Some of the stimuli arise within the organism, while others come from its environment and impinge upon it. When the quantity or quality of these stimuli or the reactions to them exceed certain limits, injury results.

The exogenous stimuli may be divided roughly into living and non-living. The living ones are primarily the lower forms of life which are potential parasites, saprophytes or commensals. For most animals, the first of these is the most important from the point of view of defense mechanisms. The non-living exogenous stimuli fall into three main groups: (1) mechanical, which includes local or generalized pressures that may result in trauma; (2) forms of energy, as temperature changes in environment, radiant energy, electricity; (3) chemical—this group includes the foodstuffs, water and gases taken into the body as well as other materials ingested or administered as drugs and poisons. Some of these substances cause injury by their absence (vitamin or other food deficiencies).

The endogenous stimuli are to a large extent concerned with the development of the organism and its functioning in the developed state; the reactions to them are merely expressions of the animal's activity as a living organism and are concerned with the maintenance of optimal conditions for life under a given environment. This includes

those mechanisms which act to keep the internal environment constant.

It is obvious that for any given individual at a given physiological level, different intensities of the same stimulus would produce different results. Further, the same stimulus administered to the same individual at different physiological levels would elicit still different results. Such differences become apparent when a population of similar individuals, each of which is at slightly different reactive levels because of individual heredity or previous experience, is exposed to the same stimulus of given intensity. In this case, variations in reaction will appear, although for most of the individuals the results are likely to fall within certain relatively close limits.

On the other hand, a given stimulus when applied to a variety of different species may call forth an extreme variety of effects, ranging from very intense reactions to those which are not detectable.

Stimuli may act over a long period; they may be repeated from time to time and call forth the same or different reactions. Stimuli may act locally or over the whole organism calling forth local or generalized reactions, or both.

A break in the mechanism by which a normal stimulus is mediated may result in injury. Or, the reacting mechanism may be insufficient to cope with a stimulus of greatly increased intensity and again injury may result. Hence, many physiological reactions may gradually merge into pathological ones. Is there any way of delimiting them?

It may be helpful to view this problem as exemplified in three experiments, involving different reactive mechanisms in which a gradual increase in the strength of the stimulus is applied. These will be presented in somewhat oversimplified form. A good example is the maintenance of the acid-base balance in the mammalian blood stream. If a small amount of acid is added to the blood stream, the alkali reserve falls but the pH remains practically the same because of the buffering action of the blood. Is this

a defense reaction? If much more acid is added continuously to the blood, a point will soon be reached when the alkaline reserve of this tissue is greatly diminished and other mechanisms will be called into play, as hyperventilation, increased acid elimination, mobilization of alkali from the other tissues. Is this stage injury and are the resulting processes defense reactions? Or must we wait until even more acid has been added to the blood in our experiment so that the subsidiary mechanisms likewise fail and the animal becomes manifestly ill and will die if the process is continued? Between each of the main stages in this experiment there is a continuous series of intermediate minor ones. Where should the line be drawn?

Let us consider another type of upset in function—the case of a diet deficient in phosphate. As long as the lowered phosphate absorption is sufficient, in the presence of large amounts of calcium, for the calcium-phosphate product to equal 40 mg/100 cc of blood, there is no injury in so far as bone is concerned. But if the amount of phosphate is decreased still further and the product falls below this figure injury will result, especially if extra need for phosphate becomes apparent as in pregnancy, and the body mobilizes phosphate from bones, or, if new bone is laid down it will be insufficiently calcified.

Still another type of experiment: Let us imagine that a very few bacteria penetrate an epithelium and reach the underlying tissue. If the cells at that point destroy the bacteria, there is no injury to the organism as a whole, even if some of the cells involved die. If more bacteria, or bacteria of greater virulence are introduced, the local cells will not be able to destroy the bacteria and other mechanisms will be called into play. This would be a reaction to injury.

Accordingly, for the ensuing discussion I shall consider the organism as being continuously exposed to exogenous and endogenous chemical and physical stimuli, many of which will be living biological systems or their products. If the stimuli are of such intensity that the mechanisms

immediately available at their site of intrusion can incorporate them into the complex interactions of the organism without disturbing it further, we may speak of reaction to stimuli of this strength as physiological. If the same stimuli are of greater intensity so that the mechanisms immediately available are insufficient and the organism must call on other mechanisms, I shall speak of these instances as injury. Or, in different words, defense may be conceived as consisting of a series of mechanisms of which the first line would be immediately available. If these are insufficient to cope with the stimulus, secondary mechanisms come into action. That is, defense consists of successive lines of reserves which become active in series after the previous defense mechanisms prove inadequate. If all of these become exhausted, the animal dies.

We may thus distinguish physiological and pathological reactions to stimuli, with the realization that these are merely extremes connected by a continuous series of intermediate stages. Somewhere between the two a limit may be set up, although few will agree on just where it should be placed. But if such a limitation is not made, it becomes impossible to speak of local and generalized defense mechanisms without including the entire physiological economy of the organism. What is meant by "immediately available" will have to be defined for each type of potentially injurious stimulus and each site of its action.

Because of their great variety it is impossible to discuss here the means used by the body in its defense against the host of injuries to which it at times is subject through increases in its endogenous stimuli or through failure of the mechanisms normally mediating them. Accordingly, I shall not consider psychic stimuli and psychic injury, or instincts, or hormone and metabolic unbalance, or hereditary defects, or the whole series of reactions by which organisms attempt to avoid injury through instinctive and volitional movements and defense weapons. Nor can I consider the defenses of the body against possible injury from many types of exogenous stimuli. For in all these cases the first lines of defense are as numerous as the

stimuli which they mediate when the dosage is not injurious.

I shall limit the discussion to an analysis of the reaction of the vertebrate and especially the mammalian body to the local and general introduction of several types of foreign substances. I shall point out how these animals, after being exposed to such an injury, are so changed that they react in different fashion to the reintroduction of the same materials.

The bodies of the vertebrates (and higher invertebrates) are composed of four main groups of tissue: (1) the epithelia which cover the body, line its cavities and form its glands; (2) the connective substance which forms the blood and blood-forming tissues, a large part of the vascular system, bone and cartilage and connective tissue between all other tissues; (3) muscle and (4) the nervous system.

As the covering epithelia separate the interior of the body from the external world, all exogenous stimuli must pass through them; they thus act as a barrier which at times is an important defense mechanism. In general, however, epithelium, nerve and muscle have but limited possibilities for defense; if they are injured sufficiently seriously they die and the nearby vascular connective tissue reacts to the injury. Eventually the damaged epithelium may be regenerated, although muscle and nerve display but limited regenerative potentialities.

REACTION TO LOCAL INJURY

The reaction to local stimulation will depend on the nature and amount of the stimulus and the defensive capacities of the local tissues. If the stimuli are of such weakness that the local mechanisms are sufficient to neutralize them, injury will not result. But if the stimulus is applied in increasing doses, a point will be reached which exceeds the capacity of the local tissue to prevent injury and secondary mechanisms of defense become effective. Such local injuries usually result in death of cells or mechanical interruption of histological continuity. As a

result of the injury, a complex series of changes occurs at that point which tends to localize the injurious stimulus, neutralize it and subsequently helps to repair the damage done. Most of this localizing reaction is carried out by the connective tissue, blood, blood-forming tissues and blood-vessels. A part of these reactions constitute the ill-defined inflammatory process. In general, it is much the same in all tissues (with a few exceptions such as the cornea, cartilage and central nervous system) and after most types of stimuli causing the injury. Although the process is fundamentally the same, it varies to the extent that different stimuli produce slightly different results and the site of the reaction will exert slight, local modifications of the process; for example, inflammation in the lungs and in the loose connective tissue.

The salient features of the simple inflammatory reaction are: First, it is a non-specific defense mechanism. Second, it is marked by the accumulation in the injured area of vast numbers of phagocytes which actively destroy part or all of the noxious material. Only a small part of these phagocytes existed in the tissue before it was injured; the circulating blood brings the great majority of them to this area as actual and potential phagocytes which arose in the blood-cell-forming tissues. Third, chemical substances pass from the blood into the injured tissue; this is not very important in most simple inflammations, although it is an important part of the specific inflammatory processes in natural or acquired immunity.

The failure of the inflammatory process to localize stimuli completely may be harmful as well as beneficial from the point of view of the animal; but in all cases it involves the whole organism in the reaction to local injuries. As we shall see, the failure to localize parasites and foreign proteins has a very important effect on the organism for, as a result of their passage into the rest of the body, reserve lines of defense react and the host becomes specifically immunized to the particular foreign agent.

The local defense reactions to aseptic injuries (those

from which parasites are absent) are the simplest to study and give a general picture of the process. The modifications that may be caused by the presence of invading parasites will be more apparent if the basic sequence of events is pointed out first. In aseptic inflammation in most instances all the injury is done at once. This is rarely the case in septic injuries in which the parasites usually multiply and continue to stimulate the defense reaction.

The reaction that follows a cut into the subcutaneous tissue, or the introduction there of a salt of a heavy metal or of a foreign protein is essentially the same in the several instances. Let us follow the local consequences of the subcutaneous injection of a small amount of a slightly irritating, vital dye into a rat. The use of this material is particularly helpful in that it aids us to discriminate between certain of the cell types involved in the reaction following its introduction. The first change after the dye is injected will be a slight swelling of the connective tissue due to the dyestuff. During the first hours of the reaction the capillaries and venules of the affected area undergo a marked dilatation. They show a marked increased permeability, and blood plasma flows into the area.

The next change in the tissue is the movement of cells from the blood into the injured area. Great numbers of heterophil granular leucocytes and of non-granular leucocytes (lymphocytes and monocytes) migrate from the vessels into the injured area which is now edematous through the accumulation of blood plasma. The heterophil leucocytes and the monocytes are each capable of ingesting certain kinds of finely divided particulate matter, while the lymphocytes rarely show this power. The migration of leucocytes of all types continues to a marked degree for the first eight to ten hours, after which it tends to diminish. It is necessary to stress the movement of the leucocytes and the liquid parts of the blood from the vessels because they represent important, easily mobilizable forces which act in defense. After their migration from the vessels, the lymphocytes and the monocytes begin to hypertrophy.

This is indicated at first by enlargement of cytoplasm or nucleus or both, with the result that a great variety of cell forms is present; these do not conform to any of the cells of the blood or normal connective or blood-forming tissues (with the exception possibly of the omentum).

During the next few hours these cells continue to hypertrophy, and at eighteen to twenty-four hours they begin to store small amounts of the dyestuff. This process of hypertrophy continues and by the end of the next twenty-four hours the inflamed tissue is crowded with masses of newly developing macrophages which have come from the hypertrophy of the blood, non-granular leucocytes and a few local macrophages; at the end of four days these new macrophages can not be distinguished from the relatively few macrophages of local origin in the inflamed area. All of the macrophages now contain large amounts of the dye.

With the start of about the fifth day, large numbers of the macrophages begin to develop into fibroblasts. During this transformation the cells become outstretched and larger and gradually lose their content of dye and of phagocytosed cellular debris. In the course of the next week the large numbers of fibroblasts now present begin to lay down collagenous fibers. The inflammatory reaction is subsiding.

If, instead of being produced by the injection of a vital dye as above—or by that of a foreign protein or salt of a heavy metal—the injury had been the result of a sterile knife wound, a defect in the tissue would have resulted. This would have been filled at first by plasma, which would then clot. The granular and non-granular leucocytes would wander into this clot. Here the granulocytes would die and the non-granular cells would hypertrophy into macrophages. Next to enter this area would be macrophages, fibroblasts and blood-vessels from the surrounding tissue. Eventually, the macrophages from these two sources in the defect develop into fibroblasts and produce dense masses of collagenous fibers; this is the scar.

What are the changes resulting locally from the injection

of bacteria and most other parasites into the tissue? The details of the reaction will vary somewhat with the virulence, type and numbers of the parasites and whether the reaction occurs in a normal or immune host. In the normal host, in most cases, the inflammatory exudation will be qualitatively the same as described above and the same cellular transformations will occur, but the percentage of granular leucocytes will be higher. Depending on the factors of virulence, etc., mentioned above, the parasites will or will not be destroyed by the macrophages and granulocytes ingesting them. This type of injurious agent differs from the non-living ones in its ability to multiply rapidly and in many cases to give off toxic substances which must be neutralized to prevent further injury to the host. In most cases of local bacterial injury, the migration of cells from the blood stream is of greater intensity and lasts longer than in aseptic injuries.

What has been accomplished as the result of this complex inflammatory reaction? In each of the above cases, the dead cells resulting from the injury have been cleared away. The defect resulting from the knife wound has been filled with scar tissue. Although much of the vital dye has been localized, most of it has diffused beyond the site of the injection and has entered the circulating blood, whence it is removed by the macrophages in the liver, spleen, etc., so that a few repetitions of the injection make it possible to "stain" the macrophages of the whole animal vitally. In the case of the foreign protein, too, only some of the material is destroyed locally, for some of it can be detected later in the blood stream and it produces changes in other organs. Although great numbers of bacteria and other parasites are destroyed in the inflamed areas, some of them or their products gain access to the general circulation, whence they tend to be removed by the macrophages, especially those of the spleen, liver, bone marrow, while those which have entered the lymph stream tend to be taken up by the macrophages of the nearest lymph nodes. This means that further lines of defense come into activity. A notable example of the incomplete localization

of invading parasites by inflammation in the invaded tissue is shown in malaria. Here a relatively insignificant inflammation ensues, the parasites do little damage, but manage to reach the blood stream, where they multiply. This is especially striking in that malarial parasites reproduce slowly as compared with most bacteria.

One of the important generalized reactions to a local introduction of parasites is the maintenance of the leucocyte level in the blood stream. We have seen that there is extensive migration of the white blood cells into the local area of injury. The movement of these cells out of the blood stream calls upon the bone marrow and lymphatic tissue reserves, and extensive changes take place in these blood-cell-forming organs in order to maintain adequate numbers of their progeny in the circulating blood. This is especially pronounced in the lymphatic tissue, where the lymphocytes are normally stored in great numbers. These cells move rapidly into the veins or into the afferent lymphatics and thence into the blood stream. This temporary depletion is quickly followed by a wide-spread multiplication of lymphocytes and the lymphatic tissue increases in amount. This is exemplified by the well-known "acute splenic tumor" of many acute infections. The demand for lymphocytes may be so great that even this rapid multiplication of them is insufficient and the lymph nodes and spleen then become depleted of their free cells and greatly reduced in size. With the subsequent destruction of the invading parasites, recovery ensues and the reserve supply of lymphocytes is again built up. These reactions can take place readily and in a relatively short time. This lability of structure of the lymphatic tissue is an important one, for it demonstrates that the lymphatic tissue all over the body is a source of easily mobilizable cells which can migrate into local inflamed areas and hypertrophy into macrophages.

It has been claimed that part of the localizing effect of the inflammatory reaction is due to clotting within the local lymphatics, thus preventing the dissemination of the ex-

traneous materials from the inflamed area. Most of the recent work does not agree that the lymphatics are blocked. In any event, it is clear that in the described cases of injected vital dyes and foreign proteins, the ensuing inflammatory reaction is insufficient to localize the stimulus.

REACTIONS TO GENERALIZED PARENTERAL STIMULI

We have just seen that substances ranging in character from vital dyes to proteins and parasites are often not localized completely by the mechanisms which come into play at their sites of introduction. These foreign substances consequently become generalized stimuli and elicit further defense reactions which tend to neutralize and destroy them. The type of generalized defense reaction will depend to some extent on the kind of substance introduced.

The host of pharmacodynamic materials acts on such a variety of tissues and organs that it is impossible to consider them in this brief general discussion. I shall consider only those types of substance which were described above as causing local injury.

Innocuous foreign particles, as colloidal carbon, are removed quickly from the blood stream by the macrophages in spleen, liver and bone marrow.

If the foreign materials are proteins or bacteria or other living parasites and are disseminated over the body by the blood stream, the resulting reaction again will depend on the type, virulence and number of the invading organisms and the degree of natural immunity of the host. With the exception of certain parasites, as those of kala-azar, which live in macrophages, organisms of low virulence and foreign proteins will be quickly removed from the blood stream and digested by the macrophages in the spleen, liver and bone marrow. In this case the reaction involves the first line of defense only, and injury, as I have defined it, does not occur. But if the parasites are of greater virulence, the macrophages, or at least some of them, will be destroyed, the parasites will begin to multiply rapidly and great numbers of new macrophages will be mobilized.

This is a reaction to injury and will occur to a small extent through the mitosis of local macrophages, through the mobilization of macrophages from the reticular cells, and largely through transformation of lymphocytes into macrophages. This process, if the demand is great enough, will cause striking changes of depletion and, eventually, overproduction of lymphocytes in the lymphatic tissue. If the parasites are present in overwhelming numbers, or are of such a nature for a particular host that the defense of the host is insufficient for the production of adequate numbers of macrophages, the animal will succumb.

One of the most important consequences of this phagocytic reaction of the body to the introduction of foreign proteins and organisms is the production of substances called antibodies. The antibodies have the ability to precipitate specifically these foreign materials (and to agglutinate them if they are cells) *in vitro*. They have the ability to render these particular foreign substances more readily phagocytosed *in vivo*. This development of antibodies is a part of the process of active immunization. As a result of this process the defensive mechanisms are specifically increased, with the consequence that the organism has an increased capacity for reacting quickly and effectively to the local or generalized reintroduction of the same foreign material.

This increased defensive capacity of the animal persists for varying periods of time and rests primarily on: (1) localization of the irritant (precipitation, agglutination) and (2) increased activity of phagocytes (opsonization).

THE LOCAL REACTION TO THE LOCAL REINTRODUCTION OF FOREIGN SUBSTANCES

Here again the reaction varies with the type of substance introduced, whether it is the same as that injected the first time, and whether or not it enters the same or similar tissue area as the first time.

(1) The reinjection of most non-protein materials into the same area as previously injected (after a sufficient time has elapsed for healing to have taken place) results in an

inflammatory reaction of the same intensity and quality as after the first injection. In this case there seems to be no change in the first lines of defense.

(2) After reinjection of the same antigen, a much more violent reaction usually ensues. This depends on whether antibodies had developed against this particular antigen. There has been a long discussion as to whether antibodies develop locally or generally or both. The majority of recent opinions is that both sources function. In the case of reintroduction of the antigen into a tissue area similar to but not identical with that involved in the first inflammation, it is obvious that the antibodies which appear with the inflammatory exudate come from other tissues by way of the blood. No matter whether the antibody has been produced locally or generally, it appears in the reinjected area of local inflammation and there unites with the antigen. In general, this antigen-antibody union results in an intense inflammation and cell death.

(3) If a living parasite is injected into the site of a previous non-specific inflammation, the macrophages aggregated at that point together with those resulting from the new inflammation enable the host to overcome the injected bacteria very quickly if they are of low virulence. That is, the immediate defense capacity of the tissue is increased because of its previous experience and a stronger stimulus is necessary to produce injury. But if the bacteria are of high virulence (as in the case of the introduction of pneumococci into rabbits by Cannon and co-workers) previous non-specific inflammation does not materially increase the local defense toward this infection.

If, however, the area had previously been injected with the same bacteria, the reinjected organisms are usually destroyed exceedingly quickly and the infection is localized. In this case the defense capacities of the tissue had been greatly enhanced by the presence of specific antibody (opsonin) which enables the macrophages remaining from the previous injection and those arising from the new stimulus to be much more effective phagocytes. The anti-

body, by agglutinating the bacteria, also tends to prevent their dissemination.

(4) The local injection of a parasite in an animal which had a previous general infection with this parasite usually results in a very rapid local destruction of the invader if antibodies have been produced in great amount during the first generalized infection. However, there are some infections in which the presence of great amounts of antibody fails to help protect the host completely. On the other hand, in those infections in which a high degree of immunity develops with the production of only small amounts of circulating antibody the local reaction to reintroduction of the organism does not always result in local destruction of the invaders. This is clearly seen in the attempt by Professor Taliaferro and myself to observe the local reaction to infections of malarial blood in normal and immunized canaries and monkeys. In the two sets of normal and immune animals the local reaction appeared identical. Here the local reaction was inadequate for the localization and destruction of the parasites, and the more generalized defense reactions came into play and prevented reinfection.

From these experiments with local reintroduction of foreign materials it is apparent that the new inflammatory reaction differs from that after the first injection. In these animals, as a result of the previous experience with the same foreign protein or parasite, specific antibodies developed against the foreign materials so that when they are reintroduced, the inflammatory reaction has become a highly specific and effective means for the localization and destruction of the foreign material. In such animals, dosages of a given bacterium which would have killed normal animals very rapidly are quickly destroyed.

REACTIONS TO REPEATED GENERALIZED PARENTERAL CHEMICAL STIMULATION

In the case of certain drugs, such as cocaine, the organism reacts to repeated doses of the substance in the same fashion each time so that the dosage of the drug need not be increased to produce a definite reaction. In the

case of repeated introduction of morphine, on the other hand, the body develops a progressive ability to conjugate the drug and render it ineffective. This means that the dosage must be continually increased to satisfy the craving which develops. In this case the body develops a defense against the possible harmful action of the drug; this is called an increase in tolerance and does not have the same basis as antibody production.

With repeated administration of a foreign protein, the body develops great amounts of antibodies to this substance. The generalized (intravenous) administration of the protein in such an immunized animal results in different reactions which depend primarily on the animal species. At some stages in the immunization process, animals of most species become hypersusceptible to the introduction of the foreign protein and suffer reactions of varying degrees of severity consequent to the union of antigen and antibody. In some species it is almost impossible to elicit this hypersensitive phase of immunization.

In the case of reintroduction of the same parasite, the macrophages all over the body ingest them extraordinarily rapidly. This is effected in part through the increased mobilization of macrophages following the first infection, and in even greater numbers through the presence of specific opsonins (antibodies) which enable the macrophages to be much more effective phagocytes. This part of the immune reaction is highly specific for the particular parasite.

An important part of this defense reaction is the mobilization of the lymphocytes of the lymphatic tissue with a subsequent hyperplasia of this tissue. This produces an easily mobilizable and transportable supply of lymphoid cells which can readily turn into macrophages. It has been shown in such different infections as those due to malaria and *B. monocytogenes* that the qualitative changes in the lymphatic tissue are much the same in the first infection and in the reinfection but that the sequence of events starts earlier and is much more rapid and intense in the immune animal.

These recent studies throw light on the so-called mesenchymal reserves of the body. These, in the adult organism, are reserve sources for (1) erythropoiesis, (2) leucopoiesis, (3) phagocytosis, (4) mechanical support by connective tissue, bone and cartilage. It is not possible to enter in great detail here into these complex problems. I have shown elsewhere that the mesenchymal reserves of the adult animal consist of two types of cells, (1) the direct descendants of the fixed mesenchymal cells which appear in the blood-forming tissues and along the capillaries as non-phagocytic reticular and perivascular cells, respectively, and (2), as free mesenchymal cells, the lymphocytes (also called hemocytoblasts). These occur to a limited extent in the bone marrow and in vast numbers in the lymphatic tissue of the lymph nodes and spleen, whence relatively small numbers of them continuously gain access to the circulating blood. Because of the close functional relationships between the lymphocytes and macrophages in the reaction to single and multiple injections of foreign proteins and parasites, Taliaferro and Mulligan have proposed the term lymphoid-macrophage system.

Although most of what I have said deals with the phenomena of inflammation and immunity, it must be kept in mind that these are not the only or the main mechanisms of defense. Although these processes result from certain types of injury and serve in many cases to neutralize or to aid in preventing the spread of the injurious agent, they are only part of the defensive mechanisms of the body. Inflammation itself is a non-specific reaction of the body to many types of local injury, usually those involving death of cells. It obviously is not "the" local defense reaction.

SUMMARY

I have tried to show that a great variety of stimuli are constantly acting on the animal body and that diverse mechanisms act to incorporate them into the functioning organism. If for reasons of quantity or quality, the mechanisms reacting to these stimuli are inadequate, injury results. In the gradation between physiological and patho-

logical reactions, I have set an arbitrary limit based on the criterion of whether the mechanisms immediately available at the site of action of the stimulus are adequate to neutralize the stimulus.

The action of many types of stimuli results in local injury and the immediate reaction of the animal is by inflammation. Through this non-specific reaction, blood plasma and large numbers of phagocytes accumulate in the injured area. The blood contributes the vast majority of these through its granulocytes—which are relatively specialized phagocytes—and through its lymphocytes and monocytes which soon hypertrophy into macrophages. The phagocytes remove or neutralize much of the foreign material.

The simple inflammatory reaction is often an incomplete barrier to the extension of the harmful agent. As a consequence, further defense mechanisms of more generalized type become active. If the injurious agents are foreign proteins or parasites, specific antibodies are formed against them—presumably by the macrophages—as part of the process of immunization.

Simple inflammation differs from immune inflammation, for in the latter case the organism through its natural or acquired immunity reacts specifically and in most instances very efficiently in the neutralization of noxious factors.

The generalized reintroduction of a foreign protein or parasite into an animal which is specifically immunized results in most instances in a relatively quick neutralization of the noxious agent through the efficient interaction of macrophages and antibodies. In many cases, particularly if the dosage of the stimulus is small, these reactions may be so efficient that it may be difficult to demonstrate the reactivity of the host. Here it is apparent that a stimulus, which in a non-prepared host would have required an extensive series of defense mechanisms, is now overcome by the first line of defense of the immunized animal.

The lymphatic tissue acts as a mesenchymal reserve in

the simple and immune, local and generalized reactions by furnishing large numbers of lymphocytes which readily turn into macrophages.

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A RELATION BETWEEN BREED CHARACTERISTICS AND POOR REPRODUCTION IN WHITE WYANDOTTE FOWLS¹

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It is to be expected that originators of new breeds of domestic animals, in their efforts to establish breeds differing from those already existing, will occasionally adopt as a breed characteristic some exceedingly undesirable mutation. In a recent review (Hutt, 1934) there were discussed 31 lethal mutations in domestic animals. Of these no less than five had been incorporated as breed characteristics to produce Dexter cattle, Creeper (Scots Dumpie) fowls, the hairless dog, Crested ducks and the grey Tzourcana and Shiraz sheep. To this list can now be added two more such cases. In the Dark Cornish (Indian Game) fowls, the more short-legged birds, according to Landauer (1935), are heterozygous for a lethal mutation different from that in Creepers. Lienhart (1937) found that in the so-called rosy Gier pigeon a sex-linked lethal gene has been incorporated to turn the unwanted ashen-headed birds into the creamy-headed type which the fancier desires.

Evidence for another case of low reproduction associated with breed characteristics is presented in this paper. Poultry breeders have long complained of White Wyandottes as being inferior to other breeds in their capacity to reproduce, but actual proof of this contention has not hitherto been advanced. Experimental demonstration of breed differences in reproductive capacity requires: (1) that the breeding birds compared should come from several different strains of the breeds to be compared; (2) that they be random samples with respect to their abilities to reproduce; (3) that the environmental, dietary and mating conditions be practically identical for all birds; (4) that the eggs be incubated under identical conditions; (5) repetition

¹ Number 10 in the author's series "Genetics of the Fowl."

of the test for several years, with changing stock, till conclusive results have been obtained.

THE DATA

All these requirements have been met by the National Official Pedigree Breeding Station operated from 1929 to 1935 by the Lancashire Utility Poultry Society at New Langton, Lanes., England, and also by the Northern Ireland Pedigree Poultry Breeding Station at Hillsborough, County Down, which has been conducted since 1933 by the Agricultural Research Institute of Northern Ireland.

The object of both stations was to provide official supervision over the pedigreeing of chicks hatched from fowls which had previously qualified, by laying 200 or more first-grade eggs in an official laying test, for registration by the National Poultry Council. At each station housing, feeding, management and incubation were alike for all birds. Records were kept of the numbers of eggs incubated, infertiles, dead-in-shell, chicks hatched, crippled and weak chicks, losses during rearing, culls eliminated and of healthy chicks reared. These are summarized in the annual reports of the two stations. The plan of operation was practically the same in Northern Ireland as in Lancashire.

Nowhere in the published reports of these enterprises is there any suggestion that a comparison of breeds was contemplated. However, their records do provide exactly the data needed for comparisons of breeds with respect to efficiency of reproduction.² The analysis in this paper is restricted to White Leghorns, Rhode Island Reds and White Wyandottes, which together comprised about 90 per cent. of all birds entered. To emphasize the adequacy of these birds as samples of the breeds compared, their sources for representative years are given. In 1931 pedigreed progeny were produced at the Lancashire station by

² The author is indebted to Mr. R. W. Hale, secretary of the institute, for information facilitating analyses of the records from Northern Ireland, and to Mr. E. Collier, secretary of the society, for records and information concerning the Lancashire station.

Leghorns from 24 different farms, Rhode Island Reds from 17 farms and Wyandottes from 18 farms. In Northern Ireland in 1936 Rhode Island Reds were sent in from 28 different farms, White Leghorns from 12 and White Wyandottes from 30. All the birds at this station came from Northern Ireland, whereas in Lancashire the birds came from all over England and Scotland, but, with the exception of a single entry in seven years, not from Ireland. The data from both stations thus make the sampling of the breeds much more adequate than could those from either station alone. Altogether about 15,000 eggs from each breed are involved.

TABLE 1
SUMMARY OF REPRODUCTION IN THREE BREEDS*

Breed	Number eggs incubated	Infertile Per cent.	Dead in shell Per cent.	Chicks Per hatched Per cent.	Weak chicks Per cent.
<i>Lancashire (7 years)</i>					
White Leghorn	12,521	20.0	19.1	64.8	6.5
Rhode Island Red	9,887	17.0	18.6	67.6	5.3
White Wyandotte	10,966	31.4	21.6	53.8	11.8
<i>Northern Ireland (5 years)</i>					
White Leghorn	2,271	10.7	25.4	66.8	8.2
Rhode Island Red	7,236	9.0	20.0	72.3	3.9
White Wyandotte	4,842	19.3	21.4	63.4	8.1

* The percentages are based as follows: Infertile eggs—upon the numbers incubated; dead-in-shell—upon the numbers left in after removal of the infertiles and dead embryos on the eighteenth day; chicks hatched—upon the numbers of eggs incubated; weak chicks—upon the numbers of chicks hatched.

To save space the actual numbers of infertile eggs, chicks hatched, etc., for each breed each year are omitted and a summary is presented (Table 1) showing the percentages for the critical factors by which the efficiency of reproduction can be measured. This covers the seven years during which the Lancashire station was operated and the five years from 1934 to 1938 for Northern Ireland. The data for 1933 at the latter station are omitted because the numbers in that year were small and the conditions of incubation and candling were not the same as in later years.³

³ Infertility appears somewhat higher at the Lancashire station than it really was because candling there was done only on the eighteenth day, at which time all eggs recognized as containing dead embryos were lumped with

ANALYSIS OF THE DATA

In determining the significance of the differences between breeds, tests for heterogeneity showed that there was too much variation from year to year to permit all the data for any breed from one station to be lumped together as a homogeneous sample to which the standard error of a percentage could be applied. Environmental conditions evidently varied enough to raise the hatchability for all breeds in one year and to lower it in the next. Accordingly, comparisons were made by Student's pairing method so that at each station one year's figures for any one breed were compared separately with those for each of the other two breeds in that same year. For example, in determining the difference between Wyandottes and Leghorns in fertility there was a series of seven comparisons in Lancashire and five in Northern Ireland. In Table 2, where the mean differences are shown, the values for p give the probabilities that differences as great as those observed might have arisen by chance.

TABLE 2
DIFFERENCES BETWEEN BREEDS

Comparison	Infertile		Chicks hatched		Weak chicks	
	Mean difference	p	Mean difference	p	Mean difference	p
<i>Lancashire</i>						
W. Wyand.—W. Leghorn .	+ 10.6*	.014	- 12.5*	< .01	+ 5.1*	< .01
W. Wyand.—R. I. Red . . .	+ 13.2*	< .01	- 11.8*	< .01	+ 6.7*	< .01
R. I. Red—W. Leghorn . .	- 2.7	.29	+ 1.1	.71	- 1.6	.18
<i>Northern Ireland</i>						
W. Wyand.—W. Leghorn .	+ 9.7*	< .01	- 4.0	.48	- 0.3	.82
W. Wyand.—R. I. Red . . .	+ 10.3*	.019	- 8.2	.07	+ 3.8*	.016
R. I. Red—W. Leghorn . .	- 0.7	.63	+ 4.2	.28	- 4.1*	< .01

* Statistically significant differences.

The number of chicks hatched per hundred eggs incubated was consistently lower in Wyandottes than in the other two breeds. The differences of 11.8 and 12.5 per cent. were statistically significant in the Lancashire data, the infertiles in a class called "unfertile." In Northern Ireland "addled" eggs containing embryos dying before the eighteenth day were classed with the dead-in-shell. This difference in procedure explains the differences between the two stations with respect to frequencies in these two classes.

but those of 4.0 and 8.2 per cent. in Northern Ireland were not. At the latter station the number of Leghorn hens was small, varying in the five years from 12 to 16. With such numbers, one or two poor sires could affect adversely the record of reproduction for the year. The Leghorn records fluctuated more than those of other breeds. In 1938, when the number of Wyandottes also dropped to 14, their eggs actually hatched better than those of both the other breeds. It is not surprising, therefore, that the breed differences in chicks hatched were not more significant statistically at this station.

The data in Table 1 show no consistent breed differences in dead-in-shell, although with the exception of the Irish Leghorns the Wyandottes were a trifle higher in this respect than the others. The factor affecting most the numbers of chicks hatched was the infertility, which at both stations was higher in Wyandottes than in the other two breeds (Table 2). The differences, varying from 9.7 to 13.2 per cent., were all highly significant statistically. Apart from this analysis the consistency of the differences

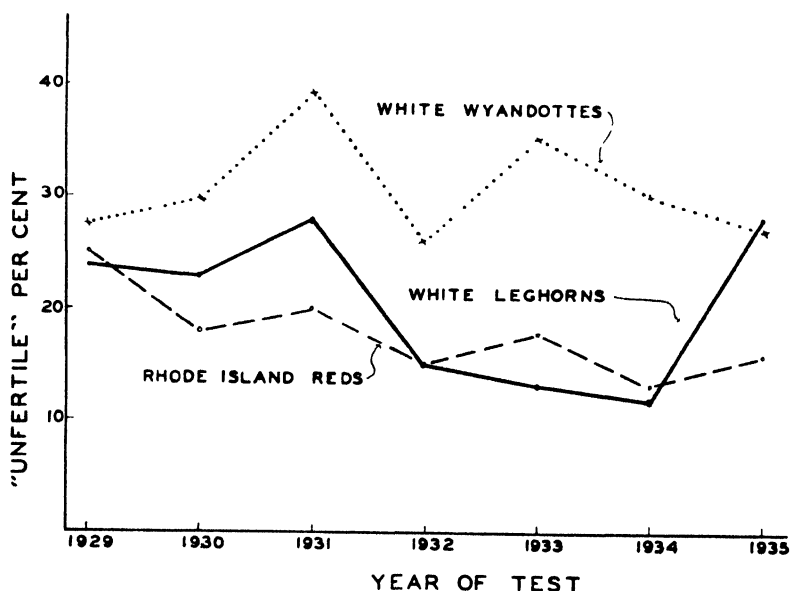


FIG. 1. Incidence of "unfertilized" eggs in three breeds at the Lancashire station over seven years.

from year to year is convincing. Fig. 1, where data for the "unfertiles" at the Lancashire station are plotted, shows that the Wyandottes were consistently higher in this respect than both other breeds in every year except 1935, when the sample of Leghorns was too small to prevent considerable fluctuation.

The "weak chicks" include cripples and defective chicks of various kinds. In Lancashire the proportion of these in Wyandottes (11.8 per cent.) was higher than in the other two breeds by statistically significant differences. The consistency of these from year to year is shown in Fig. 2. Similarly in Northern Ireland there were significantly fewer weaklings among Rhode Island Reds than in Wyandottes, but the Leghorns had slightly more. This may have resulted in part from the smaller sample of the Leghorn breed at that station. It is noteworthy that the proportion of weak chicks was lower at both stations in Rhode Island Reds than in the other two breeds.

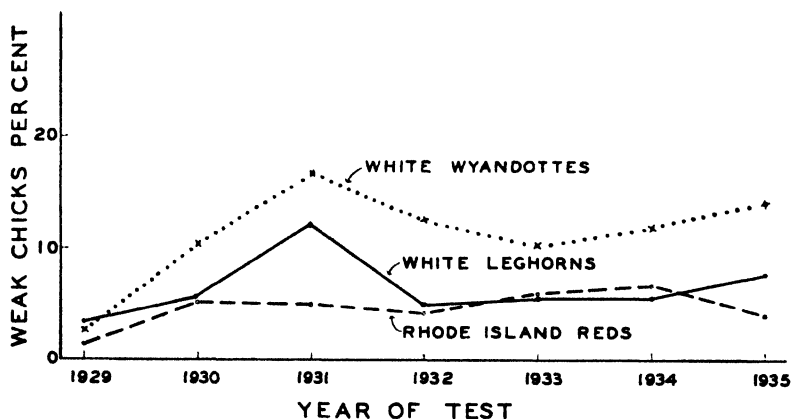


FIG. 2. Incidence of weak chicks in three breeds at the Lancashire station over seven years.

The White Leghorns and Rhode Island Reds did not differ significantly in any of the measures of reproduction, with the doubtful exception of weak chicks in Northern Ireland. This makes all the more striking the consistent and significant differences between these two breeds and the Wyandottes.

DISCUSSION

Something associated with the genetic constitution of the White Wyandotte is evidently incompatible with maximum efficiency in reproduction. Since neither station made a sharp distinction between fertile eggs and those containing embryos which died early, the question is still open whether the difficulty lies in failure of the eggs to be fertilized, or whether fertility is normal but early embryonic mortality unusually high. In eggs not candled till eighteen days of incubation, those in which embryos had died during the first two days might easily be classed as infertile. For that reason the data under discussion, though indicating that fertility is abnormally low in White Wyandottes, do not exclude the possibility of early embryonic mortality being equally responsible for subnormal reproduction in that breed.

No reason is apparent why a breed subject to low fertility should also have a high proportion of weak chicks. The differences reported may have resulted as much from preferences of the persons grading the chicks as from anything else, and, since the defects are not specified, the breed differences in "weak chicks" should probably not be considered as definitely established.

There are indications that some fowls infected with *Salmonella pullorum* tend to have more than average mortality among their embryos. The possibility of differential breed susceptibility to that organism being indirectly responsible for the differences in reproduction is eliminated by the fact that at both stations only birds proven by blood test to be free from that organism were admitted.

Since White Wyandottes are recessive white, it is possible that a lethal gene linked with that color, for which some evidence was found by Dunn (1923), may be a factor affecting reproduction in that breed. However, other recessive white breeds and varieties have not been subject to the same suspicion as White Wyandottes. In this connection it is of interest to recall the popular (but unconfirmed) belief among breeders of Shorthorn cattle that white heif-

ers are "shy breeders." Whether or not colored varieties of Wyandottes are as poor breeders as the white ones remains to be determined.

Rhode Island Reds seem the most efficient of the three breeds, so far as reproduction is concerned, since in that breed infertility, dead-in-shell and weak chicks are lower at both stations than in the other two breeds. However, the differences between Reds and Leghorns are in general much smaller than those between either of them and the Wyandottes and are less consistent.

Not all White Wyandottes are poor breeders. The fact that some males and females have good capacity to reproduce has led to considerable controversy over the reputed shortcomings of the breed, with some poultrymen stoutly maintaining that reproduction in Wyandottes is fully as good as in other breeds. It is evident, however, that the average for the breed is low. The condition responsible may be a pleiotropic effect of some gene, or genes, determining the characteristics of the breed, or it may result from genes linked with those making the breed. In either case it is as much of an inherited trait as are the rose comb and white color by which the White Wyandotte is distinguished.

Recognition of this fact may be responsible for the waning of the breed's popularity in the United States, but till recently it has been a favorite in the British Isles. Of all farms sending registered hens to the Lancashire station during its last three years, 1933-35, 30 per cent. had White Wyandottes. In Northern Ireland, 32 per cent. of all farms entering birds during the three-year period, 1935-37, sent that same breed. However, the writer has been advised by Mr. R. W. Hale that the proportion of Wyandotte entries in the official laying tests in England, Scotland and Northern Ireland declined from a high figure of 28 per cent. in 1932 to 17 per cent. in 1937. This probably indicates recognition of the breed's shortcomings. White Wyandottes make unexcelled table fowls, and strains capable of good egg production have been established. It is to

be hoped that the cause of the subnormal reproduction may be discovered and eliminated from a breed which is otherwise deservedly popular.

SUMMARY

Analyses of records of official pedigree breeding stations in Lancashire and in Northern Ireland show that the ability of White Wyandottes to reproduce is subnormal in comparison with White Leghorns and Rhode Island Reds. A total of about 15,000 eggs for each breed is involved, and the samples came from many different farms. The numbers of chicks hatched per 100 White Wyandotte eggs incubated were 12.5 and 11.8 less than for White Leghorns and Rhode Island Reds, respectively, at one station and 4.0 and 8.2 less at the other. These differences apparently resulted chiefly from infertility, though early embryonic mortality may also have been a factor.

The subnormal reproduction is attributed to the pleiotropic action of some gene, or genes, determining the characteristics of White Wyandottes, or to linkage of deleterious genes with those making the breed.

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SUBSPECIES, HYBRIDS AND SPECIATION IN *DROSOPHILA HYDEI* AND *DROSOPHILA* *VIRILIS*¹

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INTRODUCTION

THE work on *Drosophila melanogaster* is too well known to warrant any general discussion of the value of *Drosophila* as a genetic tool. Yet from time to time there have been certain objections raised to the practicability of using this animal for studies on the nature of evolutionary processes. One early objection had to do with the very small size of *Drosophila* chromosomes. To-day the giant salivary chromosomes of the Diptera are recognized as better than large plant or amphibian chromosomes for cytological study. Their enormous size and intricate visible pattern have made possible the development of a new cytology at a level of analysis impossible with other material.

A second objection sometimes raised was that mutations in *Drosophila* were of the "bottle" variety. And indeed with the perfection of the x-ray technique for manufacturing mutations wholesale and the almost universal application of this technique by the majority of *Drosophilists* in a variety of experiments purporting to deal with the factors of natural evolution there may have been some reason for this criticism. We do not mean to minimize the importance of x-rays in studying the structure of the chromatin. But it has seemed a rather invalid assumption that x-ray changes are necessarily parallel in quality to

¹ The major part of the work herein reported was done while the author held a fellowship under the Rockefeller Foundation at the William G. Kerekhoff Laboratory of Biology, California Institute of Technology. The author wishes to thank Dr. T. H. Morgan and his staff for the generous use of facilities in that laboratory, Dr. Th. Dobzhansky for suggestions concerning study of hybrid sterility, Dr. M. Steggerda for material collected and especially Dr. A. H. Sturtevant for his generous help and sound criticism on matters dealing with taxonomy.

and merely differ in quantity from natural mutational processes. Recent evidence from several sources points to qualitative as well as quantitative differences in x-ray and natural mutational changes. However, it is now recognized, at least by those who have worked with natural populations, that many of these are full of mutations, and that in certain places one can collect in an afternoon *Drosophila* material containing hundreds of them. In fact, for time expended the method of collecting wild flies and breeding out mutations which they carry yields more workable auto-somal material than any x-ray technique yet devised.

A third objection to *Drosophila* was that there were almost no species and subspecies crosses possible in the genus, the early case of *simulans-melanogaster* hybrids reported by Sturtevant (1919) being presumably quite exceptional. As a matter of fact relatively recent and critical study of wild *Drosophila* material indicates that many interesting and significant stages in evolution at the subspecies level are extant in this genus.

The classical work of the Morgan school on the genetics *D. melanogaster*, the ingenious experiments of Muller leading up to the discovery of transmutation by x-rays and the subsequent careful analysis made possible by this technical tool, and particularly the epochal discovery by Painter of the significance of the morphology of salivary chromosomes form a firm foundation for the more extensive work which lies ahead. The writer wishes to emphasize this point as some biologists, even some *Drosophilists*, seem to think that the *Drosophila* field has been pretty well worked, except for a detailed extension of cyto-genetic and physiologico-genetic findings via the x-ray, salivary chromosome and transplantation techniques. Actually we have at hand the material for the intelligent investigation of the causes and course of evolution in one group. This material must be handled on a scale never before attempted and by combined field, laboratory and field-experiment methods yet to be developed. The nearest approach to such studies are those made by Dobzhansky and Sturtevant (1938) and Dubinin and his co-workers (1937).

We here present some data on subspecies and subspecies crosses in *Drosophila hydei* and *Drosophila virilis* and attempt to relate them to other known cases of hybridization in the genus.

A NEW SUBSPECIES OF *DROSOPHILA HYDEI*

In June of 1938 our laboratory at Wooster received a shipment of *Drosophila* species from A. H. Sturtevant. This included four stocks of *D. hydei* collected in Yucatan, Mexico, by M. Steggerda, of the Carnegie Institution, and sent through Dr. M. Demerec to Sturtevant. On sending us this material Dr. Sturtevant called attention to the peculiar light thoracic pattern of these flies, differing from typical *D. hydei*. Dr. Steggerda has written that the material was collected either in Merida or Chichen Itza, Yucatan.

When the condition of these stocks permitted we made up many pair matings and reared some 100 F₂ cultures and examined these for mutants. Among the offspring of several of these there appeared an extreme abnormal abdomen, autosomal recessive and semi-sterile. This was the only mutant recovered by inbreeding and is one of the commonest types of mutation found in *D. hydei*. During this analysis several thousand flies were examined. As we have carried on extensive experiments of this type using wild *hydei* material from widely separated sections of this country and in several years we are in a position to know something of the normal variability of the species. The Yucatan fly was seen to differ in several morphological characters from true *hydei*. It has now been cultured in our laboratory for almost a year and continues to show this slight but constant ensemble of character differences. Furthermore, crosses with typical *hydei* stocks indicate that these characters are due to multiple factors and not to one or two gene differences. On these grounds therefore we are describing it as a new subspecies, *D. hydei yucatanensis*. There follows a taxonomic description. Only those characters in which it has been found to differ from *hydei*

hydei are included. The reader is referred to Sturtevant's description of *hydei* (1921) for an account of characters common to both subspecies.

TAXONOMIC DESCRIPTION, *DROSOPHILA HYDEI* SUBSP.

YUCATANENSIS, SUBSP. N.

Differs from *hydei hydei* in having the dark brown spots around macro- and microchaetae of the thorax much reduced in size, giving a pale, bleached appearance. This character resembles a sex-linked mutant type known as "light," but is not that mutant. A median longitudinal dark band or stripe, including the region of the middle four or five acrostical rows of microchaetae, narrower anteriorly and best seen in young flies. Eyes larger than in *hydei hydei*, being intermediate to *hydei* and *repleta* but nearer the size of the latter. Body build on the average a little toward the *repleta* type, squatter than *hydei hydei*. Type locality, Yucatan, Mexico. Cotypes in collection, College of Wooster.

On attempting a large number of crosses of *yucatanensis* with *hydei* the two forms were found to be fully inter-fertile. The salivary chromosomes of hybrid larvae were examined, and from many good preparations it was ascertained that there were no major inversions and no apparent loose pairing or other abnormality. If there are any differences in the salivaries of the two subspecies they are slight.

DROSOPHILA VIRILIS AMERICANA SPENCER

This subspecies has been described briefly by Spencer (1938) and a short account of its cytology has been given by Hughes (1939). The partial fertility of both male and female hybrids between *D. virilis americana* and *D. virilis virilis*, and the striking combination of morphological, physiological and cytological character differences between the two subspecies are of particular interest in the study of speciation in the genus. It is therefore our intention here to give a fuller account of collection records, subspecific characters and hybridization experiments than that which appeared in the brief abstract mentioned above. A detailed account of the cytology of the subspecies and hybrids is shortly to be published by Hughes, and we shall refer only briefly to facts given in the abstract cited above

(Hughes, 1939). An extended study of subspecies crosses and hybrid sterility is being made at present by Harrison Stalker at the University of Rochester.

HISTORICAL

In order to appreciate the significance of this case it will be necessary to review briefly the work which has been done on the genetics of *D. virilis*. As long as sixteen years ago Metz, Moses and Mason (1923) published a 94-page monograph on "Genetic Studies in *Drosophila virilis*." In their general introduction this observation is found:

It is believed that the only method of obtaining reliable information on chromosome evolution is by means of genetic analysis combined with cytological observation. Ideal material for such a study would be provided by a group of species satisfying the following four requirements: In the first place, it should exhibit among its members a series of different chromosome groups; secondly, the species should be susceptible to intensive breeding under controlled conditions; in the third place, one or more of the species should be favorable for genetic analysis through the study of mutant races; and lastly the species should hybridize with another and give fertile hybrids.

There follows mention of various animal and plant groups in which hybridization occurs, but in which others of the above criteria are lacking. Then comes reference to mutation in *D. melanogaster* and extensive collections of species and tests of these made by Sturtevant and Metz for possible hybrids. To quote further: "The results of these tests were all negative, however. . . . It should be noted at this point that Dr. Sturtevant has recently succeeded in hybridizing *D. melanogaster* and *D. simulans* (Sturtevant, 1920) with very interesting results. For the purposes outlined above, however, the possibilities are limited. The two species are almost identical and appear to have identical chromosome groups, and in addition the F₁ hybrids are sterile, so that only mutant genes obtained in both species can be used for comparison." This monograph reports 41 mutant types in *D. virilis*.

To-day it is of interest to note these facts in connection with the above quotations. Except for *D. melanogaster*, there are more mutant characters known in *virilis* than in

any other species of this or any other genus; over 300 mutations have been reported. The genetic maps of the chromosomes of this species have more genes accurately placed than in any other form except *melanogaster*. To quote from the abstract cited above (Spencer, 1938):

Drosophila virilis americana differs from *Drosophila virilis virilis* in the following characters: more fusiform body, darker body color, larger eye, finer eye-pile, broader carina, heavier cloud on posterior cross-vein and reddish rather than gray pupa case. In the former pupation occurs in the culture medium rather than on the sides of the culture vial. *Americana* etherizes very rapidly in contrast to the other subspecies. . . . The cross matings give a few hybrid offspring. The mating, *virilis* female by *americana* male, is more often successful than the reciprocal. When *virilis* females are impregnated by *americana* males about 2 per cent. of the eggs develop. The hybrids, both males and females, are partially fertile when crossed *inter se* or back to either parent subspecies. The F₁ hybrid resembles *americana* more closely than *virilis*.

To the above may be added this from Hughes (1939):

Metaphase plates of larval ganglia cells also show striking differences. Both the male and the female of *D. virilis virilis* have five pairs of rod-shaped chromosomes and one pair of dots. In the *D. virilis americana* female there are two pairs of V-shaped chromosomes, one pair of rods and one pair of dots. The male of this subspecies has three V-shaped chromosomes, four rods and one pair of dots. Two of the rods in the male appear to form a pair corresponding to the one pair of rods in the female. The other two rods seem to pair with one of the V-shaped chromosomes, one with each arm.

Thus not only in the genus *Drosophila* but in the very species which formed the subject of this monograph are found for the first time the combination of the four requirements deemed necessary by its authors for the reliable study of chromosome evolution. To these may be added the new tool, salivary analysis. Nor does the fact that the hybridizing forms have been described as subspecies seem in any way to invalidate the case. On this very point Dr. H. J. Muller in a personal communication to the author writes:

To gain for your and your co-workers' studies on these two forms the respect and attention which I think is due, I therefore think that *americana* should be elevated to specific rank. Surely it does not, naturally, form an interbreeding population with *virilis*, whatever the reason for that may be, because it differs in so many and such marked characters at once, without intergrades having been found in nature. Hence even though when artif-

cially bred a mixed population can be obtained, I think it should be called a different species, though one far better adapted by its crossability for the genetic study of species differences, than any hitherto obtained in *Drosophila*.

COLLECTION RECORDS

In the summer of 1936 the author set out a number of fermenting banana traps in open woodland and meadow along Sugar Creek about a mile from the village of Smithville, Wayne County, Ohio, and perhaps a quarter of a mile from the nearest farmhouse. These traps were visited once a week and flies taken to the laboratory and examined for species. On June 25 among the 97 flies taken there was one *virilis* female. On June 22, from a line of traps in the City Park, Wooster, Ohio, 125 flies were taken and among them one *virilis* male. These species collections are recorded in Table I. The *virilis* male was preserved in alcohol and on recent examination proves to be *virilis americana*. It becomes the first authentic specimen collected.

TABLE I
SPECIES COLLECTIONS OF *DROSOPHILA* CONTAINING *VIRILIS*

Species	City Park, Wooster June 22, 1936		Sugar Creek Woods June 25, 1936	
	Males	Females	Males	Females
<i>affinis</i>	35	0	4	0
<i>algonquin</i>	15	0	6	0
<i>buskii</i>	1	1	40	20
<i>functris</i>	0	0	4	0
<i>immigrans</i>	1	1	1	0
<i>melanica</i>	1	0	0	0
<i>melanogaster</i>	1	3	3	9
<i>putrida</i>	1	0	0	1
<i>robusta</i>	26	22	7	1
<i>transversa</i>	1	6	0	0
<i>virilis</i>	1	0	0	1
Totals	83	42	65	32

From the female, which had been impregnated before capture, a stock was established. Some years before we had had stocks of *virilis virilis* in our laboratory and one of the most striking characters observed was the divergence of the anterior scutellar bristles which in most species then known to us are directed backward and parallel. It was by this character that the two *virilis* flies were first spotted in the collections. The *virilis* stock was assumed to be type

virilis and taken along with other *Drosophila* material to the California Institute of Technology in September. Shortly thereafter, Sturtevant and the author compared this stock with Japanese stock of *virilis* in that laboratory. It was at once obvious that the two were quite similar but differed in a number of definite characters. Experiments were then set up to determine the extent of these differences and the possibility of the two forms hybridizing.

In the summer of 1938, early in July, the author and Mr. Harrison Stalker made collections of *Drosophila* in and near the Great Smoky Mountains National Park. From traps exposed at Gatlinburg, Tennessee, near the west fork of the Little Pigeon river among over 2,000 flies taken there were 3 *virilis americana* females. In addition to these collection records, Sturtevant has in his pinned collection of *Drosophila* a specimen taken many years ago near Chattanooga, Tennessee, by W. S. Adkins, which, although badly damaged, agrees with *americana* in carina shape and clouding of the wing vein, and probably represents the first specimen of the subspecies to be captured. Among many thousands of *Drosophila* collected by Stalker and the author in the environs of Wooster no more specimens have been secured. It would appear that the species is either a rare one in this section or not drawn to the traps by the bait exposed. The first assumption seems the more likely, as laboratory observation has shown that this fly is attracted by and feeds and breeds on the yeasted banana bait used in the traps.

(Concerning the geographical distribution of *virilis virilis* Kikkawa and Peng (1938) state: "The species is widely distributed and common in the eastern Palearctic regions, but very rare in Nearctic regions." They report collecting it at 40 different stations in Japan and Korea. Sturtevant (1921) mentions two collection records from the United States, one pair taken by himself in New York City in 1913 and a strain secured from a grocery store in Terre Haute, Indiana, by R. R. Hyde. In addition Metz, Moses and Mason (1923) mention a specimen taken in Los

Angeles. Because of the rarity of the American collections and the fact that they have all come from cities Sturtevant has suggested that the species is an introduced one. That *virilis virilis* may itself represent a complex of subtypes is indicated by certain observations. Kikkawa and Peng (1938) state: "Though no extensive study has yet been performed, the Japanese form is somewhat different from the American; namely, the former is longer than the latter in both the length of the body and of wing." They are here referring to American stocks of *virilis virilis* and not to *americana*. A Chinese stock received by us from Dr. M. Demerec crossed to *americana* with much more difficulty than did the Japanese stock used. When these were carefully compared it was found that the two *virilis virilis* stocks differed somewhat in carina shape, the Chinese being the broader.

It would appear, then, that *virilis virilis* is native to the Far East, China, Japan and Korea, and that *americana* is a subspecies which has a considerable range in the United States, though apparently a relatively rare form.

MORPHOLOGICAL CHARACTERS

Virilis americana has eyes which are larger than those of *virilis virilis*. More striking than difference in eye size is the very short pile on the eyes of the former, giving them a smooth appearance. The width of the cheek is correspondingly greater in *virilis* than in *americana*. The carina of *americana* is broad and not at all sulcate, while that of all stocks of *virilis* examined is narrower and distinctly sulcate, although as mentioned above this character is variable. The abdomen of *americana* is more fusiform, giving the fly a "stream-lined" appearance. In making some hundreds of dissections of the internal organs of females of the two subspecies it has been noted that the viscera of *virilis* have a tougher texture, not so easily ruptured when put on a stretch. Four color characters have been noted. *Americana* has a darker body color, a slightly darker eye color and a lens-shaped dark cloud on the posterior cross-

vein in contrast to a narrow cloud of uniform width in *virilis*. The pupa case of *americana* is distinctly reddish in contrast to the dark grayish cast of the other form. Counts of the arista branches on both sides for males and females show that *americana* has a larger number of these branches. See Table II.

TABLE II

COUNTS OF ARISTA BRANCHES IN *DROSOPHILA VIRILIS* SUBSPECIES AND HYBRIDS.
MEANS DERIVED FROM COUNTS OF BOTH ARISTAE OF 50 FLIES

	Parents	Males	Females
	<i>americana</i> male × <i>americana</i> female .	14.92 ± .18	15.64 ± .23
(a)	<i>virilis</i> male × <i>virilis</i> female	13.36 ± .20	13.28 ± .21
(b)	<i>virilis</i> male × <i>virilis</i> female	12.94 ± .12	13.40 ± .13
(c)	<i>virilis</i> male × <i>virilis</i> female	12.92 ± .12	12.90 ± .12
	<i>virilis</i> male × <i>americana</i> female	13.46 ± .14	13.18 ± .14
	<i>americana</i> male × <i>virilis</i> female	14.30 ± .12	13.86 ± .12
	<i>virilis</i> male × hybrid (v. male a. fem.)	13.22 ± .13	13.90 ± .19
	F ₁ hybrid male × F ₁ hybrid female . .	13.48 ± .18	14.12 ± .22

PHYSIOLOGICAL CHARACTERS

Five physiological character differences between the subspecies have been observed. *Americana*, after emergence from the pupa case, becomes sexually mature later than does *virilis*. Under the conditions of banana agar or corn-meal molasses agar culture *virilis* is more easily reared and more prolific than *americana*. With these culture media in glass containers *americana* pupates most frequently in the culture medium and *virilis* on the glass wall of the container and above the surface of the medium. See Table III. When several *virilis* flies of the same age are left undisturbed in a culture vial they tend to occupy isolated positions. *Americana* under like conditions tends to form more closely grouped aggregates. Finally, the

TABLE III

POSITION OF PUPA CASE IN SUBSPECIES AND HYBRIDS OF *DROSOPHILA VIRILIS*

Parents	Pupa touching or buried in food	Pupa on glass side of vial
<i>virilis americana</i> 3 vials	106	2
<i>virilis virilis</i> 3 vials	37	188
<i>americana</i> male × <i>virilis</i> female 6 vials, several pairs	40	2
<i>virilis</i> male × <i>americana</i> female 7 vials, several pairs	162	28

most striking physiological character noted is the etheri-

zation time. The author has at one time or another cultured an even 50 species and subspecies of *Drosophila* and of all these *virilis virilis* is without doubt the slowest to go under ether. At the other end of the series *americana* etherizes most rapidly, if we consider the time necessary to quiet the animal as etherization time. There are a few of these species which are killed by ether a little more quickly. Thus we have observed 17 morphological and physiological character differences, and it is quite possible that others might be found, particularly if internal organs were carefully studied. A thorough examination of the male external genitalia, however, has failed to show any differences here.

SUBSPECIES CHARACTERS IN THE HYBRIDS

It might seem that since there is partial fertility in F_1 , F_2 and backcrosses of hybrids of either sex to either parent subspecies this large number of character differences would offer excellent opportunity for a study of possible gene differences conditioning them and the localization of these genes by appropriate crosses using mutant markers. This seemed a particularly promising lead when it was found that in reciprocal crosses between the two subspecies the characters of *americana* proved to be dominant to those of *virilis*, except for carina width and arista count, which were clearly intermediate in the hybrids. Thus the hybrids could easily be distinguished from *virilis* but appeared almost identical to *americana*. However, it must be remembered that the majority of these characters are phenotypically less extreme than most mutants with which the *Drosophilist* works, or are of a physiological nature not easily workable. Further, a careful examination of several F_2 series indicated that most if not all of the characters amenable to studies on segregation were due to multiple factors. Thus an F_2 series showed gradations in carina width, eye size, eye pile, etc., which the author could not separate into segregating classes. We do not claim that such a study would be impossible. We do feel that at

the present stage of the *Drosophila* work it would be impracticable. If such a study were undertaken the first prerequisite would be to work out a set of culture conditions more nearly standard than those used in most laboratories. The work would require very careful quantitative measurements and statistical analysis. As the branches on the arista seemed a character lending itself to this type of study the author made complete arista branch counts on 897 flies, which totaled over 12,000 branches recorded, and included both subspecies, hybrids, F_2 hybrids, backcrosses of hybrids to both subspecies. Some of these data are shown in Table II. They indicate that there are more arista branches on *americana* and that the hybrids are intermediate. The subspecies do not differ sufficiently to make analysis of this type profitable except under very rigid environmental control. The (b) and (c) lots of *virilis virilis* were reared under more nearly optimum culture conditions than lot (a) or the *virilis americana* lot and along with F_1 hybrids show a lower standard error of the mean than do the F_2 hybrids.

Another character which is amenable to quantitative analysis is the position of the pupa case in relation to surface of the culture medium. Table III gives some data on this character in both subspecies and in the hybrids. However, such a character does not seem trustworthy for extensive analysis except under a very elaborate culture control. It is obvious from data presented in the table that the character is dominant in the F_1 hybrids, which are like *americana* and not intermediate to the two subspecies.

FERTILITY IN SUBSPECIES CROSSES AND IN HYBRIDS

When *virilis americana* and *virilis virilis* are crossed a very small per cent. of the eggs hatch, regardless of which direction the cross is made. It might be argued if the cross were made in mass culture that only a few of the females were impregnated. In order to clear up this point the following experiment was carried through. Eighteen *virilis* females were kept for several days with well-aged

americana males. These females were then isolated, placed in individual vials with no males present, egg counts made for two days, eggs given optimum culture conditions and females dissected at the end of this two-day period and the presence or absence of living sperm in the seminal receptacles recorded. The results are given in Table IV. The summary shows that the total number of eggs laid by flies giving any offspring was 336; total offspring from these flies 16; offspring from 4.7 per cent. of the eggs. The total

TABLE IV
FERTILIZATION OF EGGS IN CROSS OF AMERICANA MALES × VIRILIS FEMALES.
SEE TEXT FOR DETAILS

Female No	Number eggs laid	Adult offspring		Living sperm in females
		Male	Female	
1	39	0	1	Yes
2	45	0	0	Yes
3	83	1	2	Yes
4	74	2	6	Yes
5	accidentally killed			
6	89	1	1	Yes
7	57	0	0	No
8	85	0	0	Yes
9	71	0	0	No
10	109	0	0	No
11	91	0	0	Yes
12	35	0	0	No
13	69	0	0	No
14	70	0	0	Yes
15	51	0	2	Yes
16	102	0	0	Yes
17	74	0	0	Yes
18	78	0	0	Yes
Totals . . .	1,222	4	12	12 of 17

Total eggs from flies giving any offspring—336.

Total offspring—16. Offspring from 4.7 per cent. of eggs.

Total eggs from flies showing living sperm—881

Total offspring—16. Offspring from 1.8 per cent. of eggs.

number of eggs from females showing living sperm at the end of the experiment was 881; total number of offspring 16; offspring from 1.8 per cent. of the eggs.

It may be definitely stated that there is no marked mortality in larval or pupal stages. Under proper environment eggs hatching go on through to the adult stage. The adults furthermore are quite normal in appearance. The cross here was made to the wild Japanese stock of *virilis virilis* females. While no such elaborate experiment has been carried through using the mutant stock ruffled-interrupted-peach of *virilis* as female parents it may be stated that on several occasions vials of *americana* to this mutant

strain have given much larger numbers of hybrids than when the wild *virilis* strain was used.

In the dissection of hundreds of females both in inter and intra subspecific crosses it has been noted that generally when a female contains any sperm in the intra cross her chitinous receptacles and ventral receptacles are packed full of sperm; while when a female in the inter cross contains sperm they are relatively few in number, the receptacles not being packed full. This seems to indicate either that copulation occurs less frequently between the subspecies or that it is shorter in duration and results in the emission of a small amount of sperm. Owing to the fact that the eggs which hatch go through to the adult stage, that the hybrids are very vigorous and normal in appearance, and that certain stocks cross more readily than others it seems likely that the partial sterility is due to psychological factors leading to partial sexual isolation rather than to any incompatibility of chromosome or genic complexes.

In order to test directly whether males mated with their own females in preference to those of the other subspecies the following experiment was done. Vials were made up containing 5 *americana* males with 5 *americana* females and 5 *virilis* females, all flies having emerged within the 24-hour period previous to the beginning of the experiment. If anything, this favored cross mating, as the *virilis* females mature more rapidly than the *americana* females. After the lapse of certain time intervals females were dissected and presence or absence of sperm recorded. The results are given in Table V. A similar but less extensive experiment using *virilis* males was carried through, and results are found in Table VI.

Reference to these tables will show the partial sexual isolation of the two subspecies when confined in small vials. From this and other experiments it seems that *virilis* males do not mate as readily with *americana* females as do *americana* males with *virilis* females. Another experiment may be cited to substantiate this point. In a half pint culture bottle containing culture medium there were confined 50

TABLE V

PREFERENTIAL MATING. AMERICANA MALES (5) × AMERICANA FEMALES (5)
AND VIRILIS FEMALES (5) PER VIAL. FEMALES DISSECTED AT
INTERVALS AND PRESENCE OF SPERM RECORDED

Vial No.	Time after start of experiment	Americana females		Virilis females	
		sperm	no sperm	sperm	no sperm
1	56 hours	0	5	0	5
2	106	0	5	0	5
3	135	0	5	0	5
4	156	4	1	2	3
5	156	5	0	3	2
6	156	4	1	1	4
7	156	2	2	2	2
8	156	5	0	2	3
9	156	5	0	5	0
10	156	4	0	1	2
11	156	3	0	1	3
12	156	4	0	5	0
13	180	4	1	5	0
14	180	0	5	0	5

Note. Vial 14 very moldy; shows that condition of food affects mating

americana males with 50 females of each subspecies. At the end of 16 days surviving females were dissected and examined for presence of sperm. A similar experiment was

TABLE VI

PREFERENTIAL MATING. VIRILIS MALES (5) × VIRILIS FEMALES (5) AND
AMERICANA FEMALES (5) PER VIAL. FEMALES DISSECTED AFTER
180 HOURS AND PRESENCE OF SPERM RECORDED

Vial No.	Time after start of experiment	Americana females		Virilis females	
		sperm	no sperm	sperm	no sperm
1	180 hours	2	3	5	0
2	180	0	4	5	0

carried out simultaneously, using *virilis* males. The results are given in Table VII.

TABLE VII

PREFERENTIAL MATING. 50 MALES WITH 50 FEMALES OF EACH SUBSPECIES KEPT
TOGETHER IN HALF-PINT CULTURE BOTTLE FOR 16 DAYS. SURVIVING
FEMALES DISSECTED AND PRESENCE OF SPERM RECORDED

Males used	Females <i>americana</i>		Females <i>virilis</i>	
	sperm	no sperm	sperm	no sperm
<i>Americana</i>	22	6	27	4
<i>Virilis</i>	12	22	38	0

One gets the impression from mass cultures of the hybrids mated *inter se* that they breed with greater ease than the original cross. However, when pair matings of hybrids of *americana* males to *virilis* females were made up, egg counts made and offspring emerging checked it was found

that some pairs showed complete sterility. Table VIII gives data secured from several pairs mated on January 31

TABLE VIII

FERTILITY OF PAIRS OF F₁ HYBRIDS FROM CROSS OF AMERICANA MALES TO VIRILIS FEMALES. MATINGS OF AGED HYBRIDS MADE ON JANUARY 31; EGGS COLLECTED FEBRUARY 3 TO 11

Pair No.	Total eggs	Total adults		Per cent. egg fertility
		Males	Females	
1	82	0	0	0.0
2	335	3	0	0.9
3	52	2	2	7.7
4	43	0	0	0.0
5	54	7	5	22.2
6	217	9	12	9.6
7	76	4	9	17.1
8	353	10	11	5.9

and from which eggs were collected over the period from February 3 to 11. These eggs were all normal in appearance and the low hatchability was probably due again to small amount of sperm, though no dissections were made. When F₂ hybrid pair matings were made the egg hatchability was even lower than with the original subspecies cross. Some of these females laid small, deformed and abortive eggs, indicating the probable segregation of abnormal factor combinations.

An experiment was run to test the fecundity (egg-laying capacity, of 12 hybrid females from cross of *virilis* female to *americana* male. Each female was placed in an egg-laying cage (Spencer, 1937) on January 31 and daily egg counts made till all females were dead. The results of this experiment show that at least some of the females have a high fecundity. The food used was banana agar, always fresh, and conditions were as nearly optimum as it was possible to supply, with temperature from 22 to 24 C. The egg counts were made daily, but in the table the data have been recorded in 11 egg-laying periods. Eggs from these females were normal in appearance, but they were unfertilized as no males were used in the experiment. The data are given in Table IX. It is not known why there was so much variability in the fecundity of these F₁ hybrid females. Nor do we have comparable

TABLE IX
FECUNDITY OF 12 F₁ HYBRID FEMALES FROM CROSS OF AMERICANA MALE × VIRILIS FEMALE. DAILY EGG COUNTS MADE BUT RECORDED
IN TABLE IN 11 PERIODS. J REFERS TO JANUARY; F—FEBRUARY; M—MARCH

Hybrid female No.	J31 to F4	F5 to F12	F13 to F18	F19 to F25	F26 to M3	M4 to M9	M10 to M16	M17 to M28	M29 to A3	A4 to A8	A9 to A16	Totals eggs	Died
1	93	152	338	264	84	79	91	39	0	1	..	1,141	April 7
2	39	54	2	11	0	17	0	0	0	123	March 13
3	296	488	335	349	344	384	447	290	290	205	50	3,478	April 16
4	51	88	61	133	6	339	March 3
5	0	82	219	121	224	112	122	118	35	35	7	1,075	April 15
6	0	193	132	0	0	1	0	0	0	0	0	326	April 20
7	143	265	285	167	860	February 25 (accidental)
8	93	94	30	78	13	19	14	0	0	341	March 30
9	17	16	70	276	188	18	2	7	0	594	March 30
10	269	565	396	158	90	40	44	17	0	1,579	April 3
11	34	32	7	15	3	91	February 28
12	44	43	0	0	0	87	February 27
Totals	1,079	2,072	1,875	1,572	952	670	720	471	325	241	57	10,034	

data on females from the two subspecies. These data are presented to show that some hybrids have high fecundity, and also to place on record an experiment on egg-laying capacity in *Drosophila* under optimum food conditions. The total of 3,478 eggs laid by hybrid female No. 3 is far in excess of the highest egg-laying record previously reported in *Drosophila*.

GENETIC CROSSING-OVER IN HYBRID FEMALES

Early observations on the salivaries of the hybrids indicated that there were major inversions in some of the chromosomes as well as much loose pairing. Such a condition would be expected to disturb cross-over ratios. In order to test this point we wrote to Dr. M. Demerec for the best multiple mutant stocks then available at the Cold Spring Harbor laboratory. Unfortunately only suitable material for testing the first and fifth of the five long chromosomes was available. When crosses of these and several single mutant stocks to *americana* were made it was found that there was a great deal of difference in the ease with which hybrids could be secured from the several crosses. The fifth chromosome stock, ruffled-interrupted-peach crossed to *americana* more easily than did any of the other mutant stocks used. In contrast some others were very difficult to cross, and in fact from some no hybrids were secured. It is quite apparent that there are factors present in the mutant stocks of *virilis virilis* which have to do with inter-subspecific fertility. Our data on crossing-over together with some secured by Mr. Robert Baxter in our laboratory indicate that cross-over values are greatly modified in those parts of chromosomes one and five which have been tested. Suitable stocks for testing of cross-over values for most of the *virilis* chromatin have not been available to us.

SUBSPECIES AND SPECIATION IN *DROSOPHILA*

The author's designation of the new *virilis* form as a subspecies has been questioned both on the grounds that

it should be given specific rank and also by those who would place it in a taxonomic rank below that of subspecies. For this and other reasons it seems pertinent to review briefly those cases in the genus *Drosophila* where hybrid formation has been found possible.

Evidence is rapidly accumulating to indicate that gene and chromosomal mutations are present in abundance in wild *Drosophila*. Thus at the primary level of inherited variation *Drosophila* populations may be thought of as fluctuating combinations of gene frequencies and chromosomal sequences. It may be asked whether point mutations or diverse chromosomal sequences ever become sufficiently established to constitute local races. The work of Dobzhansky and Sturtevant (1938) proves that populations of *pseudoobscura* with varying percentages of chromosomal sequences do occur. It seems by inference probable that over the range of this and other species local populations pure for certain chromosomal sequences exist and differ in this respect from other local populations. Gene mutations have also been known to reach a high percentage frequency in local populations. For example, Sturtevant (1915) has reported a sex-linked gene, light body color, in *D. repleta*, wide-spread and making up an appreciable part of certain collections of this species. Last summer this species was recorded for the first time from Wooster, Ohio. Mr. Stalker brought in a trap which had been exposed for some time and which contained adults and larvae of *repleta* with roughly two thirds of the gene light to one third so-called wild-type. As the species is rare in this region it is quite conceivable that a small local population could be carried through the winter by a few light individuals and become homozygous light. Spencer (1938) has reported high frequencies of bobbed genes in various *hydei* populations. But these several gene and chromosomal changes appear to be perfectly interfertile with the so-called wild-type and are thus subject to contamination even after pure races are established.

There is apparently little difference between the estab-

lishment of a local race differing from the type of the species by one gene and the isolation and fixation of a local race differing in an ensemble of characters conditioned by several mutational changes. This, in fact, appears to be the evolutionary status of *D. hydei yucatanensis*, a form perfectly interfertile with *hydei hydei*, but differing from the latter by several inherited characters not associated with hybrid sterility or gross chromosomal rearrangements. In a similar category may be placed the subspecies of *affinis* and *athabasca* described by Sturtevant and Dobzhansky (1936). In both of these cases the descriptions of subspecies are based upon slight inherited color differences found in different parts of the geographical range of the species.

Let us now consider cases in which there is a range from partial to complete hybrid sterility in intercrossing forms. As described above the cross *virilis virilis* \times *virilis americana* gives hybrids which are partially fertile in both sexes though the initial cross is made with difficulty. The principal isolating mechanism when crosses are attempted appears to be psychological. In nature geographical isolation is also a factor. The hybrids of both sexes are partially fertile. Next in order as regards hybrid sterility is the cross between the morphologically identical Race A and Race B of *pseudoobscura* (Lancefield, 1929). The hybrid females are partially fertile when crossed back to males of either race; the hybrid males are completely sterile. Finally there are the crosses between *melanogaster* and *simulans* (Sturtevant, 1919), *pseudoobscura* and *miranda* (Dobzhansky, 1935), and *azteca* and *athabasca* (Sturtevant and Dobzhansky, 1936) which give completely sterile hybrids. Further in these cases there are disturbances in the sex-ratio of the hybrids and certain morphological abnormalities in one or both sexes. Thus we have in nature in this genus a series of cases ranging from variants at the single gene mutation and chromosome inversion level, through completely interfertile forms differing by several morphological characters, to

restricted interbreeding types with partial fertility of hybrids of both sexes, complete sterility of hybrids of one sex, and finally complete sterility of hybrids of both sexes. In addition the genus contains groups of species which although intersterile are so nearly alike morphologically that they can only be distinguished by careful attention to minute morphological characters.

Any attempt to classify these forms in an ascending hierarchy of genetic relationships is bound to meet with difficulties. To suppose that the cases mentioned here can be fitted into any static scheme involving fixed levels of variety, race, subspecies and species is to lose sight of the dynamic nature of the process which brought them about. To simplify we may consider only those cases where two forms cross with some difficulty and give hybrids which show some degree of sterility. Conceivably these may be seriated in regard to: differences in morphology and physiology; in psychology and sex reactions; in salivary chromosome structure; in gonial chromosome groups; in hybrid fertility; and in geographical and ecological distribution. An attempt at this has been made in Table X. Obviously this is only an approximation. For one thing the variation of strains within the race or subspecies in reactions to another race makes it impossible to seriate accurately even for one category. Furthermore, the author's information has been gleaned partly from published descriptions and no really accurate comparison can be made without first-hand manipulation of the living material. It seems clear, however, that within this genus evolution has progressed and is progressing along diverse lines, rapidly in one respect and more slowly in another.

In conclusion, we may question the validity of calling the two forms of *pseudoobscura* races, *melanogaster* and *simulans* or *miranda* and *pseudoobscura* species, or *americana* and *virilis*, *hydei* and *yucatanensis*, *athabasca* and *mahican* subspecies. After all, what does it matter? It might be well for Drosophilists to formulate some work-

TABLE X
SERIATION OF CASES OF HYBRIDIZING FORMS IN DROSOPHILA. MOST SIMILAR TYPES AT THE LEFT GRADING TO MOST
DIVERSE TYPES AT THE RIGHT

Differences based on					
Morphology and physiology	<i>Pseudoobscura</i> A <i>Pseudoobscura</i> B	<i>Miranda</i> <i>Pseudoobscura</i>	<i>Athabasca</i> <i>Azteca</i>	<i>Melanogaster</i> <i>Simulans</i>	<i>Americana</i> <i>Virilis</i>
Salivary chromosomes	<i>Melanogaster</i> <i>Simulans</i>	<i>Pseudoobscura</i> A <i>Pseudoobscura</i> B	<i>Americana</i> <i>Virilis</i>	<i>Miranda</i> <i>Pseudoobscura</i>	<i>Athabasca</i> <i>Azteca</i>
Gonial chromosomes	<i>Athabasca</i> <i>Azteca</i>	<i>Pseudoobscura</i> A <i>Pseudoobscura</i> B	<i>Melanogaster</i> <i>Simulans</i>	<i>Miranda</i> <i>Pseudoobscura</i>	<i>Americana</i> <i>Virilis</i>
Sex reactions	<i>Pseudoobscura</i> A <i>Pseudoobscura</i> B	<i>Miranda</i> <i>Pseudoobscura</i>	<i>Athabasca</i> <i>Azteca</i>	<i>Americana</i> <i>Virilis</i>	<i>Melanogaster</i> <i>Simulans</i>
Hybrid sterility	<i>Americana</i> <i>Virilis</i>	<i>Pseudoobscura</i> A <i>Pseudoobscura</i> B	<i>Miranda</i> <i>Pseudoobscura</i>	<i>Melanogaster</i> <i>Simulans</i>	<i>Athabasca</i> <i>Azteca</i>
Geographical distribution	<i>Melanogaster</i> <i>Simulans</i>	<i>Miranda</i> <i>Pseudoobscura</i>	<i>Pseudoobscura</i> A <i>Pseudoobscura</i> B	<i>Athabasca</i> <i>Azteca</i>	<i>Americana</i> <i>Virilis</i>

ing principle for classifying, but perhaps the ink would hardly be dry when a new case outside the rules would be discovered. Apparently we are at last in a position to see species, subspecies, racial and varietal lines breaking down before the advances of field and laboratory study of evolution in the making.

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SHORTER ARTICLES AND DISCUSSION

CRASPEDACUSTA AGAIN—

EARLY in September of the present year several persons called the writer's attention to the occurrence of jellyfish in the water of Cheat Lake. The lake was at once visited, and half a dozen living specimens were obtained, after some effort, with the aid of a student, James Hunter, and his rowboat.

It was reported by other observers at the time that "thousands" of the medusae had been seen in the same vicinity. Another collection of seven living specimens was brought to the laboratory by another student a few days later.

My identification of the medusa as *Craspedacusta sowerbii*, the only fresh-water species found in the United States, was verified by Dr. Waldo L. Schmidt, of the United States National Museum.

Cheat Lake is an artificial body, formed by the damming, about thirteen years ago, of the Cheat River by a power company. It is about seven miles long and has an average width of about one fourth mile. The surface of the water is 860 feet above sea level. The specimens in question were found near the Ice's Ferry Bridge, seven miles northeast of Morgantown.

This is the fourth report of *Craspedacusta* from West Virginia: Professor P. C. Bibbee reports it from Wood County, on the western border of the state and from Mercer County on the southern border; Professor P. J. Zuccherro reports it from Fayette County in the center of the state; and the present find is in Monongalia County on the northern boundary of the state.

According to Schmidt,¹ the medusa has been found in twenty states of the Union, in the District of Columbia and in the Canal Zone.

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STUDIES ON THE CHEMOTHERAPY OF CANCER IN MICE DERIVED FROM GENETIC PRINCIPLES

It is the purpose of this paper to outline, in brief, the development of cancer research as conceived some twenty years ago and executed uninterruptedly up to the present time. The original

¹ AM. NAT., January-February, 1939.

investigation of the production of uniform stocks of experimental mice was undertaken for two reasons: (1) to eliminate or to standardize biological variability that unquestionably influences the onset and fate of malignancy within the organism and (2) to provide a supply of animals that give rise to spontaneous tumors. It may be recalled that this work was started when spontaneous tumors in mice were very rare and even before the induction of tumors by pure chemicals had been placed on a mass production basis. There is still a prevalent feeling among cancer investigators that the study of the origin of spontaneous tumors in experimental animals may lead to clearer concepts of the fundamental nature of the disease than some of the present investigations on the induction of tumors.

The use of standardized stocks of mice made possible the analysis of susceptibility and resistance to transplantable tumors. It was possible to demonstrate that these phenomena were strictly inherited according to the fundamental genetic laws as postulated by Mendel. Two concepts on the nature of cancer were formulated from this work, as follows: (1) the cancer cell is controlled by an intrinsic or genetic constitution which underlies its physiological behavior and (2) the genetic constitution of the tumor cell has deviated, by a process analogous to somatic mutation, from the genetic constitution of the tissue of the body from which it has originated. That is, it is reasonably clear that the origin of cancer, brought about by a diversity of physical and chemical agents, such as x-rays, severe burns, benzpyrene, o-aminoazotoluol, etc., or by unknown agents that lead toward spontaneous cancer, is, in the last analysis, brought about by a response or reorganization of the genetic constitutional factors within the cell which control the physiological functions of that cell. If this concept be valid, based upon indirect rather than direct experimental evidence, then certain agents known to have an effect on genetic phenomena within cells ought to or may have an effect on the origin and fate of neoplastic cells. Among those agents which have been demonstrated to have an effect upon genetic phenomena within cells is temperature. It has been shown that temperature, under which *Drosophila* (the common fruit-fly) are kept, has a pronounced effect upon crossing-over, that genetic phenomenon within cells upon which the concept of the linear order of the genes and the architecture of the germ plasm is based. The experimental regulation of different temperatures to localized

parts of an organism in which the heat mechanism is very carefully controlled, such as it is in mammals, is more difficult than it is in a cold-blooded animal such as *Drosophila*. Consequently, the best one can do is to analyze or to standardize some of the component agents which bring about the regulation of temperature. Among these agents is hemoglobin, the oxygen carrier to the tissues of the body. The amount of circulating hemoglobin is, at any one time, a resultant of the pouring into the blood of new supplies of the material and the using or dissipation of the material during its physiological functioning.

In collaboration with Dr. T. H. Werner, an investigation was set up in order to determine shifts of hemoglobin per unit of time in those individuals known by past experience to differ in their intrinsic ability to give rise to, or not to give rise to, spontaneous tumors of the mammary gland. It was clearly shown that there was a close correlation between hemoglobin readings per unit of time and this intrinsic genetic propensity to develop spontaneous tumors. Eight different genetic strains of mice were used; 3 of these developed tumors of the breast very early in life (A, C₃H and D); 2 strains developed tumors at advanced ages—22 to 26 months of life (C and CHI); and 3 were, under the conditions of the laboratory, relatively resistant to the same disease of cancer (N, JK and CBA). The 3 susceptible-to-carcinoma strains gave fundamentally the same type of curve (a gradually decreasing type beginning at 150 days of life); the 3 relatively resistant strains gave a uniformly different curve per unit of time (a rapidly increasing type up to the time when spontaneous tumors were occurring in the susceptible strains, 350 to 450 days of life); while the 2 strains of an intermediate degree of susceptibility to the onset of malignancy gave an intermediate type of hemoglobin shift per unit of time between the highly susceptible and the relatively resistant strains. It was concluded that this fundamental shift in the hemoglobin level of the blood may be an index of the physiological aging process within the organism and may be part of the genetic constitution of the mouse (quite distinct from the genetic constitution of localized cells of the body) in relation to the onset of malignancy that may be determined by genetic factors.

The lowest curve of the 3 resistant-to-cancer strains was obtained with the CBA mice. The highest curve of the 3 susceptible-to-cancer strains was found in the A mice. Consequently, if this

difference could be proven to be statistically significant, then the trends in hemoglobin with the other classes where a greater variation in readings is present, is, more than likely, significant. When opportunity arose to repeat the hemoglobin work under entirely different laboratory conditions, the CBA and A strains were selected. This work was done in collaboration with Dr. L. D. Francis. The conclusion has recently been verified from this work done at Yale, that there is a precocious drop in hemoglobin in those mice which are susceptible to give rise to spontaneous tumors of the mammary gland.

Another conclusion of the original work, done in collaboration with Dr. T. H. Werner at Bar Harbor, was that there is not only a fundamental difference in the shift of hemoglobin level per unit of time between those mice which are genetically susceptible to give rise to tumors and those which are genetically resistant, but also another difference in the shift of hemoglobin correlated with the number of hours the mouse has been separated from food. That is, a young individual of any strain of mouse has an ascending type of curve for hemoglobin shift between 2 and 6 hours following separation from food. With advancing age, this curve shifts to a descending type. This shift is probably part of that of the diurnal rhythm of hemoglobin which is of frequent occurrence in mankind, the significance of which is quite unknown. Now, it was determined that the change from the ascending to the descending type of curve took place at quite different chronological ages in mice of the different strains. This change is somehow or other correlated with a susceptibility to give rise to spontaneous tumors, since it occurs earlier in life in mice of those strains which were susceptible to spontaneous tumors. It may also be a reflection of the physiological aging process which is unquestionably a powerful force in the origin of spontaneous tumors.

Since a standard oatmeal mixture was the diet upon which all experimental mice were kept at that time, an attempt was made to investigate the influence of certain chemicals added to this otherwise basic diet. It was found that by the subtraction of salt from the basic diet, an elevation of hemoglobin within a few hours was obtained. Similarly, the addition of certain of the essential oils, particularly oil of lemon (citral), oil of gaultheria and oil of vanillin, produced an elevation of hemoglobin within a few hours on human subjects.

The nature of this hemoglobin shift has not as yet been deter-

mined. One or more of several conceptions may be valid, as follows: (1) the shifts of hemoglobin may be due to dehydration phenomena within the body, (2) the availability of food materials to the tissues of the body may be diminished (it is known that oil of gaultheria and other of the essential oils slow up enzymic activity, particularly in the alimentary canal), or (3) the oxidation-reduction potentials of the tissues may be impaired or altered (the essential oils, particularly the ones used, contained, in addition to other chemicals, the aldehydes, which are powerful reducing agents). Perhaps some other explanation of the phenomenon observed is the true one.

The next step was to add small amounts of the common essential oils to the diet of those mice which were susceptible to the origin of malignancy. When this was done, it was discovered that the onset of cancer could be delayed, and in some cases actually prevented, by adding either (1) citral or (2) oil of gaultheria or (3) oil of thyme or (4) oil of allspice to the otherwise normal control diet. Several other of the essential oils have also been tested with varying degrees of success. Since most of the naturally occurring essential oils are mixtures of several components, it was necessary to restrict the development of the research to one. For this reason, the oil of gaultheria, containing, as it does, only 5 identified components, was selected.

Malignancy can be influenced by adding small amounts of the natural oil of gaultheria to the diet of a mouse after the spontaneous tumor has originated. No such effect could be obtained when the synthetic redistilled methyl salicylate was used. Further work disclosed the fact that the active agent in the natural oil of gaultheria which brought about liquefaction of spontaneous tumors was contained in the low boiling point fraction of that oil. The other two boiling point fractions obtained by fractional distillation of the true oil were, like the synthetic methyl salicylate, quite ineffectual in bringing about similar changes in tumors. Since heptyl aldehyde is contained in the low boiling point fraction of the true oil of gaultheria, other mice with spontaneous tumors were placed on diets containing this agent. Pronounced liquefaction changes and complete regression of some tumors occurred under this treatment. It was found that the heptyl aldehyde alone was not as effective as the low boiling point fraction in bringing about retrogressive changes in spontaneous tumors. Consequently, another series of mice were put on a diet con-

taining 3 parts of heptyl aldehyde to one part of methyl salicylate. This combination is the most effective way so far discovered in bringing about retrogressive changes in spontaneous tumors. Twelve of 45 mice used actually completely regressed their spontaneous tumors under this experimental treatment.

Twenty dogs with various types of spontaneous tumors have so far been treated with heptyl aldehyde. Complete regression of the original tumors have already occurred in several animals.

The last experiment that will be presented at this time consists of the periodic inoculation of heptyl aldehyde into mice that are known to be susceptible to the development of spontaneous carcinomas of the lungs. Of 60 mice, that have died so far not a single one has had a spontaneous tumor. The controls, on the other hand, continue to develop lung carcinoma at the expected rate. It seems clear, therefore, that the introduction of heptyl aldehyde into a susceptible mouse, either by diet or by injection, influences the mechanism involved in the origin of cancer.

SUMMARY

The present paper consists of the logical development of a conception of the origin of cancer in experimental animals over a period of 20 years. Not all the steps involved in the development of the theory have been completely demonstrated. Concrete results in the treatment and control of cancer in both mice and dogs are now being obtained. The research has reached the point whereby the following conclusions are valid: (1) malignancy in experimental animals has been significantly influenced, (2) the control of cancer in mice, at least, may eventually be had by chemo-therapy and (3) such a control of the disease under controlled conditions must in the last analysis solve, or throw considerable light upon, the fundamental nature of cancer itself.

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A NOTE ON ABNORMAL SHAPE OF EGG¹

FULLY formed and complete eggs that deviate radically from the normal shape range are frequently encountered in farm flocks. It has been observed that hens, which have presumably entered the trapnests to lay but are prevented from doing so by

¹ Contribution No. 126, Department of Poultry Husbandry.

removing them to classrooms or into other strange quarters, frequently lay eggs which are odd in shape if time of laying is substantially delayed. These abnormalities are confined to the second egg, the first having the shape characteristic of the eggs for that hen.

That the premature removal of an egg from the duct does not influence the time of the succeeding ovulation has been demonstrated by Warren and Scott (1935). Although experiments

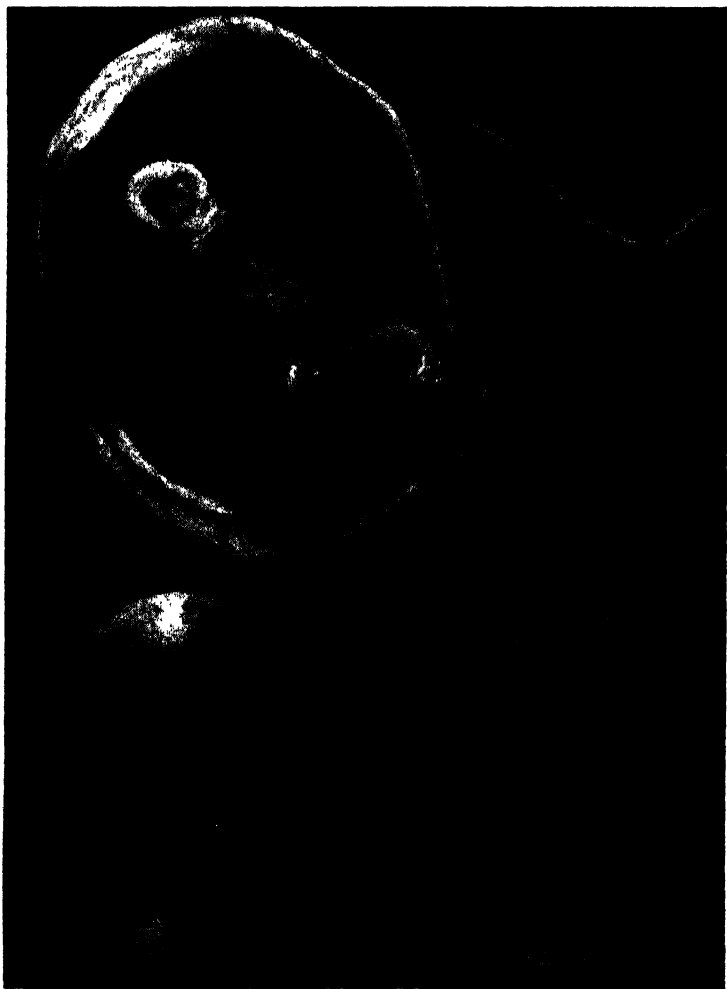


FIG. 1. Abnormal shape of egg resulting from the presence of two eggs in the fowl's shell-gland.

have not been conducted to show that a delay in oviposition likewise exerts no influence on time of ovulation we believe that such is the case, since the presence of two eggs in the oviduct has been detected repeatedly under these circumstances by palpation and confirmed at necropsy.

Usually the "held-egg" and the succeeding egg are laid on the same day and this has been interpreted by some workers as a heightened state of ovarian activity following injections of gonadotropic preparations. Not infrequently the second egg is prematurely expelled while still in a membranous state and practically devoid of calcium deposits. Although lacking a calcareous shell the egg is distinctly abnormal in shape. The area of the membrane adjacent to the shell of the "held-egg" is usually flattened. Moreover, the membranous egg retains its abnormal shape, even though the hydrostatic pressure is greatly increased by immersing in Ringer's solution. It is therefore evident that the elasticity of the shell membranes is modified shortly after they are secreted and that the abnormality resulting from the contact of the two surfaces can not be corrected by the imbibition of uterine secretion.

In view of these observations it would seem that modifications in egg shape resulting from operations upon the isthmus and uterus as reported by Asmundson and Jervis (1933) could be explained on the basis that a constriction was formed at the site of the operation which would have the same effect as two eggs in the oviduct. This would be particularly true for the isthmus whose lumen is small.

The available evidence would indicate that when carried to term chalazae formation is normal in the second egg. Their points of attachment and size are similar in every respect to those of the "held-egg." The amount of calcareous shell is, however, distinctly less than that found in the "held-egg," as shown in Table 1. Although the absolute weight of the shell of the "held-

TABLE 1
DATA CONCERNING THE AMOUNT OF SHELL IN THREE TYPES OF EGGS

	Normal egg			Held egg			Abnormal egg		
Hen	5398	5326	5392	5398	5326	5392	5398	5326	5392
Egg weight	55.6	67.0	...	60.0	67.6	62.1	59.1	55.4	61.4
Shell weight	6.1	6.5	...	6.4	7.2	6.5	4.4	4.9	5.1
Per cent. shell	11.0	9.6	...	10.7	10.7	10.5	7.4	8.8	8.3

egg" was greater than that of the normal egg the relative amounts are almost identical, and therefore the former does not

appear to have acquired more than its normal complement of shell.

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STENOTHERMY AND EURYTHERMY OF ANIMALS
IN RELATION TO HABITAT

It is in the relatively narrow range of temperatures between the freezing point of natural waters and 40° C. that most poikilothermal animals carry on their vital activities. In any one species this range may be considerably less, and vary according to conditions in the environment. If a species can tolerate a wide range of temperatures it is said to be eurythermal. If the difference between the maximum and minimum limiting temperatures is small, this condition is referred to as stenothermy. Ecologists have appreciated in a general way that stenothermal animals are characteristic of environments where temperature fluctuations are small and eurythermal animals of environments where temperature fluctuations are great, but it is only in recent years that satisfactory experimental work has begun to appear on this subject.

It is the purpose of this review to compare the range of temperatures over which normal development is possible in aquatic and terrestrial animals. In aquatic environments temperature fluctuations are small compared with those occurring on land in the temperate zone. An attempt will be made to put on a more factual basis the hypothesis that stenothermal animals exist chiefly in environments with small temperature variations, and that eurythermal animals are found under more variable temperature conditions. The data to be presented are chiefly from the literature. Many references have been omitted due either to lack of rigid temperature control or if experiments were not begun early in development. This latter precaution is necessary as resistance to heat and cold frequently increases with the stage of development. However, all cases have been included where temperature control was adequate and where experiments were

begun either at fertilization or very early in development. The normal temperatures are those permitting at least 50 per cent. survival or, in certain cases where mortality is large at all temperatures, 50 per cent. of the maximum survival.

In Table 1 the minimum and maximum temperatures, together with the range (number of degrees over which normal develop-

TABLE 1
THE RANGE OF TEMPERATURE OVER WHICH DEVELOPMENT IS NORMAL
IN AQUATIC ANIMALS

Species	Authority	Minimum °C.	Maximum °C.	Range °C.
<i>Crustacea.</i>				
<i>Leinæa elegans</i>	Nakai (1)	20.4	29.8	11
<i>Moina macrocopa</i>	Terao and Tanaka (2)	8.7	37.7	30
<i>Mollusca:</i>				
<i>Dendionotus frondosus</i>	Runnström (3)	- 1	11	13
<i>Ostrea gigas</i>	Senō, Hori and Kusa- kabe (4)	18.6	27.7	10
<i>Mytilus edulis</i>	Runnström (3)	4	16	13
<i>Mytilus edulis f. galloprovincialis</i>	Runnström (5)	8	23	16
<i>Echinodermata:</i>				
<i>Cucumaria frondosa</i>	Runnström (3)	- 1	11	13
<i>Asterias glacialis</i>	Runnström (5)	8	23	16
<i>Asterias rubens</i>	Runnström (3)	5	16	12
<i>Paracentrotus lividus</i>	Horstadius (6)			
Winter		8	23	16
Summer		16	29	14
<i>Paracentrotus lividus</i>	Runnström (5)	8	29	22
<i>Strongylocentrotus drobachensis</i>	Runnström (3)	- 1	11	13
<i>Arbacia acutituberculata</i>				
Early summer	Runnström (7)	14	29	16
Winter	Runnström (5)	8	29	22
<i>Spatangus purpuræus</i>	Runnström (5)	8	23	16
<i>Echinus esculentus</i>	Runnström (3)	4	16	13
<i>Echinus acutus</i>	Runnström (5)	8	22	15
<i>Echinocardium flavescens</i>	Runnström (3)	7	22	16
<i>Echinocardium cordatum</i>	Runnström (3)	9	23	15
<i>Echinocyamus pusillus</i>	Runnström (3)	8	23	16
<i>Psammechinus miliaris</i>	Runnström (3)	8	22	15
<i>Psammechinus microtuberculatus</i>	Runnström (5)	9	23	15
<i>Sphaerechinus granularis</i>	Runnström (7)	9	29	21
<i>Tunicata:</i>				
<i>Ciona intestinalis</i>	Runnström (3)	8	23	16
<i>Ascidia mentula</i>	Runnström (7)	8	22	15
<i>Phallusia mammillata</i>	Runnström (5)	8	23	16
<i>Ascidia aspersa</i>	Runnström (5)	8	22	15
<i>Ascidia scabra</i>	Runnström (5)	8	22	15
<i>Corella parallelograma</i>	Runnström (5)	8	22	15
<i>Pisces:</i>				
<i>Pleuronectes platessa</i>	Johansen and Krogh (8)	0	14	15
<i>Pleuronectes platessa</i>	Runnström (3)	3	14	12
<i>Ncomber scombrus</i>	Worley (9)	11	21	11
<i>Gasterosteus aculeatus</i>	Leiner (10)	8	23	16
<i>Pagrosomus major</i>	Kajiyama (11)	13.9	20.8	8
<i>Leuciscus hakuensis</i>	Kawajiri (12)	9.2	19	11
<i>Leuciscus hakuensis</i>	Nakai (13)	9.7	17.2	9
<i>Salmo trutta</i>	Kawajiri (14)	3.1	13.1	11
<i>Hypomesus olidus</i>	Higurashi and Nakai (15); Nakai (16)	6	17.5	13
<i>Calotomus japonicus</i>	Senō, Ebina and Okada (17)	19.9	27.6	9
<i>Oncorhynchus masou</i>	Kawajiri (18)	5.8	13.0	8
<i>Plecoglossus altivelis</i>	Nakai (19)	9.3	22	14
<i>Gadus callarias</i>	Johansen and Krogh (8)	- 1	10	12

ment is possible), are given for a number of aquatic animals. This range is 16° or less in 91 per cent. of the cases. The distribution is as follows: 12 per cent. have a range of 8°, 9° or 10°; 30 per cent. have a range of 11°, 12° or 13°; 49 per cent. have a range of 14°, 15° or 16°; and 9 per cent. have a range of 17° or more.

The form with the greatest range, *Moina macrocopa*, although aquatic, should probably be included with the species undergoing wide temperature fluctuations, as it inhabits very small bodies of water, the temperature of which follows atmospheric conditions closely. With this form excluded, the average of the ranges for aquatic forms listed in Table 1 is found to be 14° C.

In spite of the vast amount of published data on embryonic temperature tolerance of terrestrial animals but little has been included in Table 2. Insects, which have received the greatest

TABLE 2
THE RANGE OF TEMPERATURES OVER WHICH DEVELOPMENT IS NORMAL
IN TERRESTRIAL AND SEMI-AQUATIC ANIMALS

Species	Authority	Mini- mum °C.	Maxi- mum °C.	Range °C.
<i>Arthropoda:</i>				
<i>Cimex lectularius</i>	Gelsthardt (20)	13	36	24
<i>Cimex rotundatus</i>	Gelsthardt (20)	16	37	22
<i>Amphibia:</i>				
<i>Rana fusca</i>	Hertwig (21)	1	24	24
<i>Rana sylvatica</i>	Moore (22)	2.5	24	23
<i>Rana pipiens</i>	Moore (22)	6	28	23
<i>Rana palustris</i>	Moore (22)	7	30	24
<i>Rana clamitans</i>	Moore (22)	11	35	25
<i>Rana sphenoccephala</i>	Moore (23)	12	34	23

attention, are not especially suitable for studies of this kind. Early stages in development are frequently difficult to tell in the living egg, and consequently studies usually begin on eggs of unknown ages. Moreover, many insect eggs remain dormant at low temperatures and do not begin development until some critical temperature is passed. There is a distinct need for accurate data on the effect of temperature on development. Too often in the past investigators have been more interested in a particular theory of temperature action than in collecting accurate data to test such a hypothesis.

In Table 2 the maximum and minimum temperatures for two arthropods and six amphibians are given. It will be seen at once that there is great uniformity in the range over which normal development is possible, it being 22–25° C. in all cases and aver-

aging 23.6° C. This average is 68 per cent. greater than for purely aquatic animals. In view of the small number of cases given in Table 2 it might be well to point out that the work of Kobayashi (24) on *Musca*, Krogh (25) on *Tenebrio*, Ludwig (26) on *Popillia*, Ludwig and Cable (27) on *Drosophila*, Peairs (28) on many insects, Sanderson and Peairs (29) on many insects, Shelford (30) on the codling moth, Wadley (31) on *Toxoptera* and Wardle (32) on *Lucilia* all point to an embryonic temperature range of 20° or more for the forms studied. They have been excluded from the table because the temperature limits are not accurately determined. To these might be added additional data on amphibians (23). *Ambystoma maculatum* will develop at 3.5° and 23°; *Bufo americanus* at 10° and 30°; *Hyla crucifer* at 6° and 28°. The exact maxima and minima have not been determined in these cases, but a range of 20° or more is indicated in all. A range of this magnitude also exists in *Triton alpestris* (33), *Bufo bufo* (34) and *Pelobates fuscus* (34).

If stenothermal animals are arbitrarily defined as those having an embryonic temperature tolerance range of less than 19° C., and eurythermal animals as those having a range of 19° C. or more, we find that in 91 per cent. of the cases aquatic animals are stenothermal, and all the terrestrial and semi-aquatic species listed are eurythermal. The former live in environments where temperature fluctuations are small, and the latter where these fluctuations are large.

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THE RELATION OF GENETICS TO GEOGRAPHICAL DISTRIBUTION AND SPECIATION; SPECIATION. I.

INTRODUCTION¹

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THE examination of almost any series of superficially similar objects or phenomena reveals that they differ in degree and that they may be arranged in simple or complex *graded* order. It is a common, and perhaps logical, assumption that those units which are closer together in the gradation, or which comprise divergent lines or groups, may be referred to similar or related causes. Especially if a temporal relation parallels the seriation, evolution is demonstrated as an objective phenomenon, aside from any commitment as to cause. Some of the most complete and striking illustrations of such evolution may be found in man's arts and artifacts—his language, apparel and arts, his implements of industry and war, and, by no means least, his ideas. Here it is evident that the progressive change is due to increased experience, based on preceding trials, to wider availability of materials due to new discoveries and inventions and to different needs as more complex situations arise ("emergent" evolution). The progression here

¹ Introduction to the combined symposia given at meetings of the American Association for the Advancement of Science, the first on "The Relation of Genetics to Geographical Distribution and Speciation" at Milwaukee, Wisconsin, before Section F—Zoological Sciences, on June 20, 1939, and the second on "Speciation" at Columbus, Ohio, before a joint session of the American Society of Zoologists and the Genetics Society of America, on December 28, 1939.

does not necessarily imply any change in the inherent human capacity but merely the ability of the individual or generation to profit by its own experience or by that of those which preceded it. In this it differs from organic series, where the superficial picture is strikingly similar, but in which each link in the chain is organically (materially, genetically) related to the one that precedes and the one that follows.

The early Greek philosophers were by no means blind to the objective evidence of evolutionary series, but their ideas as to causes were well at the bottom of the evolutionary scale of ideas on evolution. As Newman has aptly put it in speaking of Osborn's "From the Greeks to Darwin":

Professor Osborn studies the evolution of the evolution idea as a biologist would investigate the evolution of a species, using all of the available sources of evidence at his disposal. The fragments of ancient writing and the crude imaginings of early natural philosophers are the fossils of the evolution idea, many of them ancestors of modern principles; fragments of ancient or discarded ideas that still persist, though irrelevant to modern thought, are the vestigial structures that proclaim kinship between the past and the present; parallelisms between the development of ideas in the minds of independent thinkers do not prove plagiarism, but indicate common descent from the same ancestral ideas.

The earliest thoughts on evolution of which we have record are mostly vague speculations involving little more than the idea of change in the universe, as opposed to fixity. Apparently the "evolution question" was not a burning one in ancient Greece, as it came to be quite literally in Europe a few centuries later, but was a matter of academic concern primarily among a small group of philosophers and their disciples—the intelligentsia of the times. During the Dark Ages the theologians were in the saddle and if there were any original thoughts on evolution they remained discreetly unexpressed. With the revival of learning in the sixteenth century evolution again came to the fore and the battle about it, while shifting front from time to time, has since been continuous. As attention has become centered on the question of *how* evolution works we are prone to

forget that even to-day a great mass of people, indifferently or variously educated, are still convinced that it does not occur at all. Everything was made as it is or, at best, was created out of hand from time to time. True fundamentalism has no cross-fertility with evolution—it is a species strictly apart, and because of its survival into these times may be considered the lingula of the evolution-ideology family. It appears as the creation myth in the oldest strata of biological speculation and remains specifically unchanged through the centuries.

Evolutionary thought—the doctrine of progressive or at any rate of orderly or seriated change—early diverged into two “varieties” on the basis of “cause.” Empedocles apparently was satisfied to leave the process largely to chance, but later philosophers, and particularly Aristotle, impressed by the niceties of adaptation in animals and plants, felt there must be an underlying predetermined plan. Evolution proceeded, it is true, according to laws of nature, but these were but the workings of intelligent design. Thus the rival schools of materialism and of divine guidance, resulting in the age-long “struggle of science and religion,” got off to an early start. With biologists this has to-day become a question of mechanism *vs.* vitalism. Mechanism, though that is a poor name for it, holds that life and evolution might be explained according to the same laws and forces operating in the inorganic realm if we but knew enough about them; vitalism, on the other hand, would invoke the supervention of some wholly new and mysterious principle where life processes are concerned. While these two modes of thought are akin to attitudes on religion, in that the facts are not available definitely to prove one or the other, it is possible, fortunately, to proceed with investigation of the mechanics of evolution without awaiting a decision.

The species problem appears to-day, as it has for a century or so, to hold the master key to evolution. Darwin recognized this when he entitled his great work “*The Origin of Species.*” The necessity of change as

opposed to stability of organic forms was of course involved, and Lamarck had emphasized the importance of the environment in producing changes that were assumed to be directed and heritable. Darwin was not so much concerned with the way in which the variations arose—he rather took them for granted—as he was in how the favorable ones became selected, thus providing for the adaptation and consequent survival of their possessor. It was tacitly assumed by Darwin and by his successors that when there was considerable change it would result in new species, though geographic isolation was introduced as an important accessory. It is generally conceded that so long as the diverging forms remain in contact and are capable of interbreeding freely, specific difference can scarcely be attained. There is, therefore, not only the question of the *origin* of divergent forms but the equally important one of their *maintenance* as separate species.

Until early in the present century biologists were handicapped by lack of knowledge of the details of relationship of one generation to another which have been revealed by the rapid advance of genetic and cytological research. It has become apparent that variations, now generally called mutations, may arise in a variety of ways and that, in addition to gene change, chromosome number and arrangement (e.g., autopolyploidy) may be potent factors not only in originating new characters but in producing cytological “breeding barriers” which may be even more effective than geographical barriers in the attaining of species rank.

It will be noted that while this discussion has been around the species, no definition of it either as a concept or as a fact has been attempted. Probably no entirely satisfactory definition has been made or is possible until we have a clearer idea of what is involved. For practical purposes the taxonomist will of necessity use a different concept from one the geneticist might formulate on theoretical grounds or on the basis of breeding

or cytological tests not ordinarily available to the taxonomist.

Discussions of evolution had reached the point of being relatively sterile and largely academic until the newer knowledge of the cytological and genetic mechanics of heredity exercised its revivifying influence. The older methods of observation and speculation have since given way to definitely directed experimentation and logical exposition. Units of inheritance have been identified and located and by their manipulation and alteration a considerable measure of control over variation has been achieved. Varieties exhibiting greater differences than those ordinarily distinguishing related species in nature have been produced and maintained. Finally, by indirect manipulation of the chromosomes, new forms have been produced which meet that further strict requirement of true species, namely that, while themselves fully fertile, they are more or less infertile with their nearest relatives. The discovery of cases in which the same process has occurred under natural conditions means that the problem of the origin of species, at least in plants, has been partially solved. This is, however, apparently only one of several methods by which specific differences may arise and become genetically isolated. The intensive research on these problems that is now being prosecuted in many laboratories is abundantly attested by the following symposia on speciation.

SPECIATION OF FISHES¹

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THE view-point of a naturalist will be expressed in this contribution. Even when laboratory experiments are recited, attention will constantly be focused on the origin and differentiation of forms in nature. The ecological aspect of speciation is considered no less vital than the genetic, since the environment is as indispensable in evolution as are the changing gene ratios. In this view a genetic type, whatever may have been its origin, has not speciated until through multiplication and inbreeding it has developed into one or more discrete populations, which have met and survived the strenuous ordeals of competition and adjustment in raw nature (Jordan, 1922). The production of a breed of chickens, or the isolation in the laboratory of a mutant strain of *Drosophila*, is not speciation—however significant the study of such processes may be in the analysis of the origin of species.

My treatment of the subject will reflect the thoughts not only of a naturalist but also of a specialist on one group, the fishes. Students of other groups, however, should find analogies which will render the remarks applicable to the animals which they study. Time does not permit the pointing out of these analogies, nor even a comprehensive treatment of speciation in fishes. Except for occasional examples, actual data can not be fitted in.

I

Recent studies in the systematics of fishes emphasize how intimately and how consistently the vital features of the environment are correlated with many if not most of the differences which characterize local races, subspecies, representative species and even genera.

¹ Read at a symposium on "The Relation of Genetics to Geographical Distribution and Speciation," before Section F—Zoological Sciences, American Association for the Advancement of Science, Milwaukee, Wisconsin, June 20, 1939.

The tie-up between specific environmental features and the characters of the evolving forms is particularly obvious in parallel variation, which therefore calls for attention. Critical studies of the variations of forms throughout their range are bringing to light an increasing number of character gradients or "clines," which are definitely associated with specific environmental gradients.

Attention has long been directed to the common tendency of fish forms of cooler waters to have a larger number of segments than those living in warmer waters. Thus, the number of parts increases toward the north, as also toward the open sea—for increased salinity has about the same speciation effect as decreased temperature. Forms of warmer water, and in the sea those of brackish water, typically have deeper bodies and larger heads than those of colder or more saline waters. I have indicated that increased numbers of somites, vertebrae, scale rows and fin rays and changes in body form tend to be correlated with retardations of development, whatever may be the cause for the slackening of the pertinent stage of development (Hubbs, 1926).

In some groups the reverse gradient in the number of certain meristic structures, as related to environmental temperatures, is the rule. In respect to the number of dorsal rays, for example, the Middle American viviparous cyprinodonts demonstrate a decrease not only toward the north but also toward the uplands or *tierras templadas*. Our recent systematic studies show that this latitudinal and altitudinal decrease in dorsal rays is a general phenomenon, and our breeding experiments prove that even small average differences in the number of rays are genetic.

Correlated gradients in characters of fishes and in the environment now appear to dominate the systematic ichthyological picture in the western fresh-waters of America. Whereas the ranges of the forms, as given, have been limited almost solely by the boundaries of

stream systems, our recent studies emphasize differentiations that are correlated with ecology rather than geography. Forms inhabiting smaller streams contrast with those living in large, swift rivers. The creek forms live in streams with a steep grade, but generally avoid fast water by frequenting the pools between riffles or by hiding in quiet pockets under and about boulders. The swift-river forms are more constantly facing the stress of current. In obvious adaptation, they tend to be larger and much more stream-lined fishes, with more terete bodies, sharper entering wedges and, particularly, with much slenderer caudal peduncles and longer and more falcate fins; and often with more rays in one or more fins or with smaller scales (which provide a smoother surface). Within *Formenkreisen*, series showing successive stages in these modifications are being demonstrated, and the same sort of gradients are repeated in different stream systems. Similar gradients, consistently in the same direction and with the same ecological correlations, have been identified in a considerable proportion of the more ubiquitous stream-inhabiting fishes of the West: in various species of *Catostomus* and *Pantosteus* among the suckers; of *Gila*, *Richardsonius*, *Lepidomeda* and *Apocope* (= *Rhinichthys*) among the minnows; of *Cottus*, the fresh-water sculpin; and probably of *Prosopium* and *Salmo* among the salmonoids. Parallel speciation correlated with current is obvious in other parts of the world, though less clearly pictured. The same character differences, depending more on consistency than on degree, in different cases define races, subspecies, species and genera.

Parallel speciation under the control of the environment has become strikingly evident during our recent studies of the fishes of the isolated waters of the arid American West. In the flowing streams, as already mentioned, the local forms of any species tend to have a stream-lined form, long and falcate fins and other characters enabling the fish the better to resist the current.

The riffle inhabitants are designed for bottom-life and bottom-feeding, having flat ventral surfaces and arched dorsal contours, and inferior, more or less horizontal mouths. In the isolated springs these special modifications of form are lost. The mouth tends to become larger and more oblique, fitted to engulf food organisms living at various water levels, rather than to grub around the stones of the riffles. In correlation with a general freedom from predation, the populations become dense and the sense organs reduced. Thus, the barbelled minnows lose their barbels, and the lateral line becomes obsolescent. The scales tend to drop their orderly arrangement and their deep imbrication, and to develop radii on all fields. In three originally unlike genera the terminal modifications along this speciational line have become so nearly molded into the same form, that they falsely appear to be very closely related.

Among the cyprinodont fishes, the desert-spring isolates tend to lose their pelvic fins. The loss has taken place in several regions and in several groups, and seems to be another example of the general tendency for animals to lose structures which are not of critical value under the environmental conditions to which they are subjected.

Parallelism is also evident in the evolution of lacustrine forms, fitted by their long and close-set gill-rakers and related changes in mouth structure to feed upon the rich plankton of lakes.

In studying the fresh-water fishes of the East Indies, I have been impressed with another character gradient which is repeated in different species, particularly of Cyprinidae. Races inhabiting the relatively sterile waters of mountain lakes and creeks tend to be elongate, terete, small-headed, small-eyed, and small-finned. Those of the lowland rivers, which abound in fertility, have deep though compressed bodies, large heads and eyes and expansive fins. These differences seem to be related to nutrition, and strikingly parallel the features which distinguish wild carp from the highly selected, fast-growing

Edelkarpfen of the German fish culturists. As usual in parallel speciation, the characters of the organism appear to have been brought into a harmonious adjustment with the usual or average features of its habitat. This relation seems to allow of normal development and life under the fluctuating environment with no undue strains or dangers. Successive adjustments of this sort provide a simple explanation for the origin of graded series of races (Hubbs, 1928).

Fishes of the very silty streams, as of the Great Plains, often if not generally, have smaller eyes but compensatingly better developed tactile organs than their congeners of clearer water. Particularly striking in the fishes of such darkened waters are the sense organs which appear to serve as current detectors. (This problem is being studied by my student, George A. Moore.)

The increased development of lateral-line sense organs and the increased flabbiness of deep-water fishes constitute a well-known and long-appreciated character gradient. The uniformity with which this gradient has been developed along many lines of evolution, and the strictness by which it is correlated with the environment, are truly remarkable.

Parallel modifications for life in lesser depths are perhaps of more significance in the study of speciation, since the connecting links are less liable to be broken. In many *Formenkreisen* there are littoral forms (races or species) which are boldly colored with generally brown tones, have thick skeletons and spines and small eyes. Their representatives in the twilight zone of moderate depths are paler and redder (therefore inconspicuous where red light rays are lacking), have thinner bones and spines, larger eyes and often better developed sense organs. Offshore pelagic derivatives are characterized in general by their metallic blue colors, terete bodies and small fins.

The more or less complete degeneration of the eyes in truly hypogeal fishes is emphasized as a virtually uni-

versal phenomenon by my recent discoveries of five new blind fishes in the caves and artesian waters of North America, and by a summary treatment of the known cave and blind fishes (Hubbs, 1938). Something that is inherent in life in absolute darkness not only permits but forces the degeneration of the eyes and of pigment. The hypothesis is often proposed that the loss of these structures in cave fishes is an adaptation in that it conserves energy in a habitat where food is scarce, but this idea fails to explain why the nutrition-bathed entoparasites have likewise lost their eyes and pigment. I have suggested that such degenerative speciation may be the result of repeated mutations, in the total absence of selection, and have computed some possible rates of speciation on this basis.

Parallel speciation among fishes is nowhere more strikingly shown than among the lampreys (Hubbs, 1925; Hubbs and Trautman, 1937: 9-14). Again and again the parasitic lampreys have evolved into non-parasitic forms, through the elimination of the adult-feeding stage, the reduction in adult size and the degeneration of the intestine, teeth and mouth. Extreme end stages are confusingly alike, though clearly of diverse origin. The evolution of the non-parasitic or brook lampreys has been correlated with life in small streams, where a suitable food supply in the way of large fish is scarce or seasonal.

The tendency for such correlated gradients in characters and in the environment to be repeated again and again in the same direction, must be basic to an understanding of speciation. Any theories of species formation that fail to consider and explain the intimate tie-up between habitat and characters must remain at least incomplete. Whether the correlation results from some form of environmental determinism, or from the selection of an appropriate habitat by forms newly speciated through unadulterated genetics, does not appear to be a very real, general problem to those of us who look on

speciation as a definite interplay between the organism and its particular environment. In some types of habitats, for example, in caves, speciation no doubt continues under environmental conditions so uniform as to preclude any preadaptation followed by the selection of habitats, except perhaps in the first stages of the orthogenetic process. In other places, as in a newly invaded territory, an organism no doubt tends to settle in environments to which it is adjusted, in the sense that it shows there a high rate of survival. In general, however, the adaptation of an organism to its environment must be perfected through adjustments in characters, interwoven with the selection of suitable niches. And it may well be emphasized that the selection of a habitat to which a given form is fitted is in itself a form of natural selection, usually with some physiological speciation, and always involving the perpetuation of that which is in harmony and the elimination of that which is out of adjustment.

In the pragmatic sense, natural selection in my opinion is clearly an essential basis of speciation as well as of general phylogeny. Opponents of this view do not take into due consideration the relatively recent evidence that extremely slight selectional advantages lead in time to speciation. They neglect the overwhelming evidence for compensative adaptation when they claim that a form with a given character has no selectional advantage because other populations lacking this alleged advantage succeed equally well, even in the same habitat; the species or group less well-endowed in regard to this character will almost surely be found more propitiously endowed in some other respect. In arguing that no advantage should be attributed to very slight, perhaps only average differences in such characters as those of meristic numbers of fin rays or scales, those combating natural selection give too little weight to the evidence that such differences are the direct consequences of vitally important changes in the rate of metabolism and the rate of development (Hubbs, 1926). They side-step

the indication that multiple effects of genes or linkage bind certain characters with some unexpected features that do have survival significance. Naively, some have held that the so-called non-adaptive, specific characters may have come into expression because they are genetically associated with an increase in fecundity. How much more objective it would be to lay stress on the selectional advantage of the increased rate of reproduction as the essential change, even though it be hidden from view, and to regard the associated morphological change as a secondary consequence, which in itself might have been of neutral or even negative significance. The essential test of selection is the survival and increase of gene or genes which in any way favorably affect the chance that the individuals so endowed will survive to reproduce in the environment in which they find themselves. In considering the "survival of the fittest," more emphasis perhaps needs to be placed on "survival," less on "the fittest."

Since virtually identical speciational changes related to the environment occur so repeatedly along unrelated phyletic lines, we can not overlook the probability that a single form may independently become modified more than once into derivatives that are indistinguishable one from another. Therefore, we have as a rule no way to prove that a subspecies or species with environmentally correlated characters is not of polyphyletic origin.

II

Through a study of hundreds of specimens of natural fish hybrids representing dozens of interspecific and often intergeneric combinations in several families, I have become convinced that as a very general rule the systematic characters of fishes show blending inheritance. The same sort of inheritance seems to operate when subspecies and races of fishes have crossed. A large body of information indicates that simple Mendelian segregation very seldom results when crosses have been made between natural forms of any vertebrate group.

Geneticists may object to this view as having been based on a study of presumed hybrids collected in nature. In mating experiments, however, the same results have been obtained for a number of interspecific crosses, in several families. This has been well demonstrated, for example, in our work on hybridization in the sunfishes (Centrarchidae), in which the experimental results stand in fine accord with the observational (Hubbs and Hubbs, 1932, 1933). Data on the viviparous killifishes (Poeciliidae) are much more extensive though as yet largely unpublished. Since we have made dozens of crosses among these fishes, particularly in the genus *Mollienisia*, we feel safe in announcing the following results:

(1) The genetic behavior is similar whether the crosses are between races, subspecies, species or genera.

(2) In almost all characters the F_1 hybrids from either reciprocal cross are precisely intermediate between the parent forms, so that they may be represented by the Galtonian fraction, $\frac{1}{2}$.

(3) Backcrosses in either direction yield offspring just intermediate between the hybrids and the given parent form, so that we may designate them as $\frac{3}{4}$ hybrids on the generally discarded Galtonian system.

(4) Successive backcrossings in the same direction yield $\frac{7}{8}$ hybrids, $\frac{15}{16}$ hybrids, etc.

(5) By this method the one parent type is reconstructed, in a form indistinguishable from the original (except at times for certain color features having little or no systematic significance).

(6) In the successive backcrosses toward one parent form there are never produced definite throwbacks toward the other parent form, nor is any marked increase in variability apparent. Since thousands of fish have been obtained in such matings, we would need to argue that a very high number of multiple factors is operating—if multiple factors are involved.

(7) Similar results and conclusions come from multiple mating experiments, in which as high as five species and twelve subspecies or races have been combined in

single individuals. The consistency of characters within such broods is amazing; the variability seems to be no greater than in the stocks of any of the constituent forms. The characters behave in such strict conformance with the Galtonian scheme of inheritance that one can, for instance, compute rather precisely the number of dorsal rays in the final multiple hybrid by striking theoretical averages through the complex mating chart, starting only with the known average value of each form as it is introduced into the multiple matings. Such characters as position of fins, form of body and coloration appear to show a similar type of inheritance.

(8) F_2 hybrids, on the average, follow the same fractional system of inheritance, and remain consistently intermediate between the parent forms, when the whole organism is considered, though showing a considerable increase in variability when any one character is analyzed. In apparent contrast with the results of successive backcrosses and of multiple matings, a limited number of multiple factors seems on this basis to be involved.

(9) For those interested in the type of sex determination and in fertility relations, it may be stated for *Mollienisia* and related genera that the male hybrids are the more fertile in interspecific and intergeneric crosses, and that graded degrees of fecundity exist. In general the fertility of the hybrids in each sex is directly proportionate to the closeness of relationship between the parent forms, with irregularities that seem to be due to the differential adaptability of the forms to aquarium conditions. Incidentally, we find in these observations no evidence to support the hypothesis advocated by Shull, that sudden breaks in fertility relations are essential in speciation.

In stressing the general absence of simple Mendelian segregation in the systematic characters of vertebrates, I do not overlook the evidence that many examples of unit factors have been demonstrated in this group. In the poeciliid fishes, for example, the work of Gordon and Fraser (1931) has shown that many color markings exist-

ing in nature show simple genetic behavior. In general, however, the characters with such genetic basis occur hit and miss through populations without sufficient consistency to be of much systematic significance. Varied ratios of the phases may characterize populations and forms, but the presence or absence of a given phase character in itself is usually not a systematic criterion. The distinction probably does not always hold between the systematic characters showing blending inheritance on the one hand and the phase characters with a simple genetic basis on the other, but the evidence is piling up to indicate that the distinction is a real and valuable one. Let me illustrate the difference by reciting the origin of the red swordtail of aquarists. No red phase of the swordtail genus *Xiphophorus* has been taken in nature and none seems to have originated by mutation in captivity, but the red phase does occur in the related genus *Platypoecilus*, which hybridizes rather freely with *Xiphophorus*. The hybrids produced by mating a *Xiphophorus* with a red *Platypoecilus* are, in part, of an undiluted red, though intermediate in the ordinary systematic characters, such as form, number of rays, and structure of gonopodium. Red hybrids mating back to *Xiphophorus* produce three-quarter hybrids of which, however, a certain proportion is red. One or two more backcrossings then reconstruct the swordtail, in all respects other than the red clothing.

Nor do I overlook the evidence that many systematic characters in plants and insects have been proved to have a simple Mendelian basis. Perhaps the high number of non-duplicated chromosomes characteristic of vertebrates is in part responsible for the blending inheritance generally shown by the systematic characters.

In dealing with speciation among the vertebrates, much of uncertainty and incompleteness must be attached to any theories or discussions which do not take into consideration the evidence that blending generally features the inheritance of racial, subspecific, specific and generic characters. The biological significance of this circum-

stance has been illuminatingly treated by Sumner (1932), and is now receiving the consideration of Dice, in connection with their intensive studies of speciation in the mice of the genus *Peromyscus*.

III

The close correlation between environment and characters is particularly well shown where speciation is released from the deadening effect of biotic saturation. In the great rift lakes of Africa, and in some other geologically recent lakes over the world, radiative adaptation has almost explosively filled the newly created niches (Worthington, 1937; Herre, 1933, etc.).

Evidence is accumulating, particularly from our analysis of the fish faunas of the isolated waters of the West, to indicate that speciation with environmental correlations may be unexpectedly rapid, when the swamping effects of outbreeding are eliminated. In several isolated waters, evolution has proceeded far enough to produce new subspecies within what appears to be a few hundred years, at most, a few thousand.

IV

Years ago the Scandinavian ichthyologist, Smitt, emphasized the tendency toward parallelism in the observed differences between species, between sexes and between growth stages, but the time was apparently not ripe for a general appreciation of this significant phenomenon. In our own recent work these parallelisms are becoming very apparent. In 1927 I pointed out that the environmentally correlated differences between forms typically involve varied degrees of completeness of the processes of growth and differentiation. Thus systematic differences are definitely linked with age variations. It will hardly need to be stated that sexual dimorphism to a large degree can be brought into the same picture.

As an example of the multiple correlations in differentiation, I will cite the situation in the Ameiuridae, or North American fresh-water catfishes. Aside from a general tendency for a northward decrease in the number

of anal rays, most of the racial differences within the species involve an increase toward the north in the thickness and depth of the body, along with a decrease in the length and roughness of the fin spines. These variational gradients are repeated in the same direction in different species of *Amciurus* and in species of other genera in the family. The same differences distinguish females from males, and young from adults. Species as well as races are involved, since, for example, *Amciurus nebulosus* differs from *Amciurus melas* in the same way that the southern race of either species differs from the northern one, or the female from the male, or the young from the old. We thus find a striking and general parallelism in the variational gradients, which may be thus expressed:

A. nebulosus → *A. melas*
Southern race → northern race
Female sex → male sex
Young stage → old stage.

In respect to coloration we find a reversed gradient, in that *Amciurus melas* is the more juvenile, as are the northern races and the males, so that the gradient formula for this character becomes:

A. nebulosus → *A. melas*
Southern race → northern race
Female sex → male sex, all as before; but
Old stage → young stage.

From these empirical facts, if not from pure biological reasoning, we are led to expect that similar if not identical biochemical processes will be found to be involved in all spheres of differentiation—ontogeny, sexual dimorphism and direct environmental effects, as well as speciation. This expectation, perhaps, has often been appreciated, but I think not commonly considered so concretely and not utilized in developing a field of research—the comparative physiology and biochemistry of differentiation. The stage is now set for this basic development.

The very general and striking tendency toward parallel genetic variation, with environmental correlations, at least suggests that relatively few and perhaps relatively simple biochemical processes are usually involved in

speciation. That modifications directly induced by the environment rest on much the same biochemical basis as the genetically controlled adaptations is suggested by a number of observations. The direct modifications usually affect the very characters which show genetic adaptation, and often in the same way. For example, factors which accelerate (or retard) a given developmental stage directly induce structural modifications in the same direction as those which are correlated with inherited differences in the rate of development (Hubbs, 1926 and 1934, and papers there cited).

CONCLUSION

As a result of our recent studies on fishes, including breeding experiments as well as systematic and variational studies of natural forms throughout their range, weight is constantly being added to the theory that speciation is no aimless wandering of genes through the organic world, but rather an orderly adjustment under the rigid control of the environment.

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ECOLOGIC AND GENETIC VARIABILITY WITHIN SPECIES OF PEROMYSCUS¹

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THAT individual characters seldom are uniform throughout the whole range of any species or subspecies is well appreciated by every taxonomist. Every species and every subspecies (the geographic race of the vertebrate zoologist) varies in its characters from place to place, and more or less distinct local races or families may occur in restricted areas. A local race which is fairly uniform and distinct in its characters and which occupies a definite geographical area will probably be described as a subspecies, even if the size of the population is small. Most local races, however, are variable in their characters and are not sufficiently distinct to be recognized in taxonomy.

The variability of subspecies and species, at least in mammals, is not primarily due to a direct influence of the environment upon the individual animals, but is based upon variations in heredity. This was first demonstrated by F. B. Sumner (1932: 28-30) in his classic studies of *Peromyscus*. My own studies of the genus confirm and support Sumner's conclusions.

The considerable amount of local variability within a single subspecies is well shown by the eastern woodmouse, *Peromyscus leucopus noveboracensis*. The body dimensions and pelage color of these mice vary greatly from place to place in the eastern United States, though all are assigned to this one form (Dice, 1937: 17-31; and 1939: 3-16). Populations of these mice living in three different woodlots in the near vicinity of Ann Arbor, Michigan, differ about as much from one another in body dimensions and in pelage color as do populations of the subspecies living hundreds of miles apart.

¹ Read at a symposium on "The Relation of Genetics to Geographical Distribution and Speciation," before Section F—Zoological Sciences, American Association for the Advancement of Science, Milwaukee, Wisconsin, June 20, 1939.

The characters which distinguish some local races may have arisen through inbreeding in partially isolated habitats, with consequent chance elimination of certain genes and fixations of the remaining gene combinations (Wright, 1932: 360-362). New mutations arising in isolated or partially isolated small populations may also affect the character of the local stock, especially if the same mutation recurs frequently. Little is known, however, about the rate of elimination of genes or the rate of mutation of new characters of the sort which distinguish races, and, consequently, the effectiveness of these processes for evolution within species can not now be evaluated.

Not all the variability within species can be the result of chance alone, for it has been demonstrated that there is a tendency for the pelage color of ground-dwelling small mammals to be correlated with the soil color of their habitat (Dice and Blossom, 1937: 106-108). The pelage colors of *Peromyscus* are, at least in major features, inherited and accordingly it must be concluded that the environment in some way controls in part the heredity of these mice.

The correlation between the pelage color of small mammals and the soil color of their habitat is particularly well shown in the western arid regions, where there is considerable diversity of soil colors and usually only a scant cover of vegetation. On isolated buttes or beds of black lava the rock-inhabiting species of small mammals tend to develop dark-colored local races, while on pale-colored granite mountains or on pale-colored sands the mammals tend to be pale in color. For instance, on the Tularosa Malpais, a lava bed in the Tularosa Basin of southern New Mexico, five species of saxicolous mammals have developed black or blackish local races. These include the rock-squirrel, rock pocket-mouse, juniper-mouse (*Peromyscus nasutus*), and two species of woodrat. In addition, some individuals of the cactus-mouse (*Peromyscus eremicus*) are here dark in color, perhaps indicating that a dark-colored race of this species is now in process of formation.

The same trend for a correlation between pelage color and soil color occurs also in humid regions. The mice living on isolated sand beaches both along the Pacific (Sumner, 1917: 173-185) and Atlantic coasts (Dice, 1939: 12-15) tend to be paler in pelage color than their brethren living on dark-colored inland soils. This trend is particularly striking in the beach-mice (*Peromyscus polionotus*) of Florida and adjacent states. The mice of this species which live inland on dark-colored clays are dark buff in color, while the mice living on the pale-colored sand of the beaches are pale in color. On Santa Rosa Island, just off the coast of Florida in the Gulf of Mexico, the mice are extremely pale, almost white in color (Sumner, 1926: 149-184).

The dark colors of animals living in heavy forests and the pale colors of desert animals were noted long ago by naturalists, and numerous hypotheses have been proposed to explain this correlation. It is now clear that the dark colors of forest animals are only indirectly correlated with the heavy rainfall, with the high humidity of the atmosphere and with the density of the vegetation. In regions of heavy rainfall it will of course usually result that there is high atmospheric humidity and a rich cover of vegetation. Part of the vegetation falls to the ground each year and produces humus in the soil. The addition of the humus gives the soil a dark color, which the ground-inhabiting animals tend to match. The result is that in humid regions many of the animals are dark in color. Contrariwise, in the desert the vegetation is scanty and little humus accumulates in the soil, which, accordingly, is pale in color. Here, in correlation with the color of the soil, the animals tend to be pale in color.

The parallel production on entirely separated lava beds of somewhat similar dark-colored races of *Peromyscus* and of other small mammals, and likewise the production of pale-colored races on isolated areas of pale-colored soils can only mean that pelage color has some important relation to the life of the animals concerned. It is proba-

ble that the pelage colors of many races have been produced by natural selection, although this has not been proved in any particular instance. To our eyes, at least, most small mammals, when viewed from above in their natural habitat, appear concealingly colored.

The body size and proportions of *Peromyscus* are probably also of importance in the life of the animals, but we know little about the adaptive value of these features. We do not know, for instance, whether or not a tail which averages ten millimeters longer in one race than in another really makes much difference to the individual mouse. Certainly there is no such obvious correlation between body proportions and type of habitat as is true of color. Nevertheless, forest-dwelling *Peromyscus* have, in general, longer tails, and larger feet, perhaps correlated with their semi-arboreal habits, than do the prairie mice. Furthermore, there seems to be a tendency for desert mammals to have proportionately larger ears than forest or prairie forms. In the pocket-gophers of the Great Basin a correlation of body size with soil type and with the altitude of the habitat has been noted by Davis (1938: 338-342). It seems certain therefore, that, though still little understood, there are at least some general correlations between the body size and proportions of mammals and the character of the environment.

The above evidence indicates that the pelage color and body dimensions of small mammals are at least in part correlated with certain features of the environment. Color and size are the characters on which most geographic races of mammals are distinguished, and we are forced to conclude that, contrary to the statements in some text-books, a considerable number of taxonomic characters are influenced by the environment and are presumably adaptive.

A common ancestry for all the members of a subspecies is generally assumed by taxonomists. If a particular subspecies has now a discontinuous range, it is assumed that at some former time the range was continuous.

Thus, of the dark-colored subspecies *Peromyscus maniculatus rufinus*, which occurs in the forests of the Colorado mountains and also on isolated mountain-tops in Arizona and New Mexico, it has been assumed (Merriam, 1890: 20-21) that the range of the form was continuous during the glacial period when heavy mountain forests are presumed to have come low enough to connect together all the now isolated sections of the subspecies.

Let us now examine a particular local race within the subspecies *rufinus* to determine the conditions under which it exists. On the upper north slope of Mt. Lemmon, the highest peak of the Santa Catalina Mountains of southern Arizona, there occurs a small area of heavy Douglas fir forest. In this forest the deer-mouse is abundant, and on the humus-filled, dark-colored soil it is very dark in pelage color. It is accordingly assigned to the subspecies *rufinus* (Dice and Blossom, 1937: 48-50, 88-89). The total area on Mt. Lemmon covered by dense Douglas fir forest and inhabited by these dark-colored mice does not exceed one square mile in area. Surrounding the area of Douglas fir on Mt. Lemmon there is a belt of yellow pine forest, much more arid in type, and inhabited by a few deer-mice, which, at least at Summerhaven, are paler in color than those from the Douglas fir association, but which must nevertheless be referred to the subspecies *rufinus* (Dice, 1938: 17-18). At a still lower level on these mountains there is an encinal belt of oaks and junipers where I failed to secure any mice of this species though they probably occur rarely. Still lower, on the north side of these mountains only, there is a belt of arid grassland in which deer-mice are rare. Here the mice are a pale buff in color, and are accordingly assigned to the subspecies *sonoriensis*, which is characteristic of the pale soils of arid areas. Still lower, surrounding the bases of the mountains, is the desert belt, in which no mice of this species have been taken.

The dark-colored local race of *rufinus* living in the Douglas fir association on Mt. Lemmon constantly inter-

breeds, we may be sure, around the margins of its habitat with the paler colored local race inhabiting the yellow-pine association, and which in its turn undoubtedly interbreeds with the very differently colored subspecies *sonoriensis* occupying the nearby arid grassland belt. Under these conditions the mice living in the Douglas fir forest must constantly interchange hereditary factors with the surrounding populations. A deer-mouse can travel a distance of slightly over two miles in about two days (Murie and Murie, 1931: 203) and a deer-mouse could, therefore, if it wished, cross the whole major area of Douglas fir association on Mt. Lemmon in a single night. In the face of constant interbreeding with and immigration from the surrounding populations the local dark-colored race on Mt. Lemmon could not possibly long remain distinct except as the result of some process favoring the dark color. There is a suggestion, furthermore, that selection for dark color is in this situation rather rigorous.

If, as is strongly indicated, selection is essential to the continued existence of the dark-colored local race in the Douglas fir forest on Mt. Lemmon, does not this agency sufficiently account for the production of the race? I believe that selection, possibly aided by local mutation, actually is sufficient to produce this local race and that the hypothesis of a former extensive Douglas fir forest continuous with the forests of the Rocky Mountains is unsatisfactory. The dark-colored local race on Mt. Lemmon can not possibly be explained solely as a glacial relict.

In the Douglas fir forest on Mt. Lemmon the deer-mice were, when I studied the habitat, much more abundant than in the surrounding associations. This would result in considerable inbreeding within this local population. On the other hand, the population there is sufficiently large so that no tendency for immediate fixation of characters would be anticipated. In this flourishing population new characters would be expected to arise from time

to time by mutation, and other characters would constantly be introduced by interbreeding with the surrounding populations. Under these conditions, if we assume, as we must, that selection of some sort is operating to favor the darker colored individuals, we would seem theoretically to have satisfactory conditions for rapid evolution.

Dark-colored local races of mammals, similar to the one on Mt. Lemmon, occur on other high mountains in the Southwest, such as on the Chiricahua Mountains (Dice, 1938: 7-18), and it may be assumed that these local races have in general originated at the places where they now occur. If this be true, the subspecies *rufinus* as now recognized has had a polyphyletic origin, and the similarities of the several isolated populations making up the subspecies are due to parallel development, presumably in response to similar types of environment.

Additional evidence for parallel evolution within a subspecies of deer-mouse is presented by *Peromyscus maniculatus artemisiae* in southeastern Washington and adjacent states. This subspecies occupies the mountains and the higher parts of the Columbia Plateau north of Snake River in Washington and Idaho, and reappears on the Blue Mountains south of the river in Washington and Oregon. Snake River, however, is a barrier to the distribution of these mice which must have been in existence before the Blue Mountains were elevated. In the canyon of Snake River, at least at Lyons Ferry, the subspecies north of the river is *artemisiae*, while south of the river a different subspecies, *gambelii*, occurs. It seems certain, therefore, that the *artemisiae* of the Blue Mountains have had an origin largely or entirely independent of those assigned to the same subspecies from north of Snake River (Dice, 1939: 16-19).

Some subspecies occupy a narrow but very extended range. Such is *Peromyscus maniculatus gambelii*, which extends from interior Washington to Baja California (Osgood, 1909, pl. 1). This is an extreme range of over

1,200 miles. Along its western border, from San Francisco Bay northward, *gambelii* interbreeds with the dark-colored races *rubidus* and *oreas* and perhaps *artemisiae*. On its eastern border, north to Snake River, it interbreeds with the pale-colored race, *sonoriensis*. It is impossible to believe that *gambelii* in its long narrow range and with constant interbreeding on both sides with races of different color from itself, could long maintain its characteristics by the impetus alone of its original hereditary constitution, even if it should ever have had a uniform heredity throughout its whole range. It seems to me most logical to assume that its range and characters are determined largely by the fact that it lives in a strip of territory intermediate in type of environment between the heavy forests inhabited by the dark-colored races on the west and the arid areas inhabited by the pale-colored *sonoriensis* on the east, and that accordingly we have here a race, *gambelii*, of intermediate color and intermediate body proportions. In other words I conclude that the environment determines the characters of *gambelii* as it does those of the other races of *Peromyscus* which have been critically studied.

Mice which appear alike in pelage color and in body proportions may not necessarily have the same hereditary constitution. Tones of pelage color which are indistinguishable to our eyes may be produced by different combinations of genetic factors. For instance, the several types of dilution in the house-mouse and in other laboratory rodents may produce animals which appear alike or nearly so. Body dimensions are known to be in some laboratory animals controlled by multiple factors, different combinations of which theoretically can produce similarly appearing individuals. The local populations occupying distant parts of the range of a subspecies may therefore have very different heredity, even though the animals have the same general appearance.

The subspecies should, accordingly, in my opinion, be considered to be primarily an ecologic unit (Dice and

Blossom, 1937: 114-115). The members of a subspecies tend to occupy similar sorts of habitats, and at least some subspecific characters are known to be correlated with certain environmental features.

The taxonomy of subspecies must of necessity be based on morphological characters. This means that in mammals the color of the pelage and the dimensions of the body and its parts must form the basis of classification. The hereditary constitution of an animal or of a population, however, can not with our present knowledge be analyzed. Neither are we able to determine the ancestors of a subspecies except by comparison of its characters with those of other living subspecies. The fossil material available for most species is inadequate to give information about the evolution of subspecies. The subspecies as we recognize it in the field and in the museum therefore is composed of individuals of the same species which appear to be more or less similar in characters and which live in the same general area.

In regions of general environmental uniformity the subspecies may be expected to be more or less constant in characters, with local races poorly marked. In regions of considerable environmental diversity, on the contrary, the subspecies will likely be variable, with many local races, some of which may be strongly marked. Such variable subspecies are of course difficult to classify, especially when several opposing ecologic tendencies are involved.

The interpretation of a subspecies as being an ecologic trend is, it seems to me, more sound than the current concept of the vertebrate taxonomist, which holds that the subspecies is a unit of descent, and which implies that it also is a unit of heredity. There is good evidence that subspecies are not uniform in hereditary constitution and there also is good evidence for the polyphyletic origin of some subspecies. Accordingly, the concept that the subspecies results from an ecologic tendency more or less

uniformly expressed over a definite but not necessarily continuous area, seems most in accordance with the facts.

SUMMARY

Within every wide-ranging subspecies of animal there is a considerable amount of local variability, at least a considerable part of which is based on hereditary differences. The pelage color of *Peromyscus* and other small mammals tends to match the color of the surface soil of the habitat, demonstrating a control by the environment of part of the heredity of these animals. There also is evidence that some subspecies of *Peromyscus* have had a polyphyletic origin. Accordingly, the subspecies is considered to be primarily an ecologic unit, rather than a unit of similar descent or of similar heredity.

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SPECIATION FROM THE POINT OF VIEW OF GENETICS^{1, 2}

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ONLY a very limited discussion will be possible, in the brief space of time at our disposal, on a small sector of the general subject of "Speciation from the Point of View of Genetics." Other symposia and various authors have dealt admirably with the influence of different aspects of genetics on the subject of speciation as a part of the larger field of evolution, and one might well question whether old ideas may be clothed in new words in such a manner as to make them worthy of presentation.

Ordinarily, we take for granted that the primary factors in the evolution of species are (a) gene mutation and (b) chromosome aberrations of the various kinds. Nevertheless, despite this assumed acceptance, in principle, of the basic rôle of gene mutation in speciation, questions have been raised by various authors as to whether the differences in heritable characters within a species (*i.e.*, differences due to the action of allelic genes) can also account for the differences between species. Arguments both pro and con have been advanced, from which we may conclude that additional experimental evidence is still needed. In this discussion, it is proposed to limit the remarks to certain aspects of the rôle of gene mutation in speciation, particularly as the genes affect the chemical composition of the blood cells. A part of the material to which reference will be made has not yet been published in detail.

¹ Read at a symposium on "The Relation of Genetics to Geographical Distribution and Speciation," before Section F—Zoological Sciences, American Association for the Advancement of Science, Milwaukee, Wisconsin, June 20, 1939.

² Paper from the Department of Genetics, Agricultural Experiment Station, No. 250. The investigations in this laboratory referred to in this paper have been supported in part by grants from the Rockefeller Foundation and from the Wisconsin Alumni Research Foundation.

We must admit that experimental analyses of genetic differences and similarities between species are relatively few, in either animals or plants. Even in species which hybridize and whose species-hybrid is relatively fertile, such studies meet with difficulties. Not the least of these difficulties is that of recognizing the effects of single genes apart from the particular complex in which they are found in one species or the other. There would be an advantage, then, in using in such studies the characters produced by genes whose effects are the same, no matter what other genes are present.

An example of this kind of character is that of the antigens of the red blood cells, in so far as they have been studied within any species. The assumption is that the action of the gene on its particular antigen is more or less direct. However, a few results have been obtained, in species crosses of doves (Irwin, 1932; Irwin and Cole, 1936) and in chickens (Thomsen, 1936), which imply complementary interaction of genes having effects on the antigens of the red blood cells. It hardly need be stated that these antigenic characters are as yet detectable only by the use of immunological reagents.

No other cellular antigens have been studied so extensively as those of the human blood cells, of which there are the two well-known systems. The first of these is comprised of the characters \bar{A} , \bar{B} and \bar{O} , which are produced by triple alleles. Both \bar{A} and \bar{B} are dominant to \bar{O} (in our opinion, this seeming recessiveness of the \bar{O} character is probably due to the lack of a readily available and potent reagent for this antigen), and both \bar{A} and \bar{B} are fully expressed when heterozygous. Another pair of characters, the \bar{M} and \bar{N} , are produced by a pair of alleles, independently inherited from those producing the \bar{A} and \bar{B} antigens. Analyses of the chemical composition of the \bar{A} and \bar{B} characters by different investigators indicate that they are complex nitrogenous polysaccharides.

It becomes a question of considerable interest as to

whether these cellular characters, presumably therefore their causative genes, are present in any of man's relatives. Tests to determine these relationships have already been performed by various workers, with results as given in Table I.

TABLE I*
HUMAN BLOOD-CELL CHARACTERS IN THE BLOODS OF APES AND MONKEYS

	O	A	B	AB	M	N	MN
Chimpanzees	+	+	-	-			(+)
Gorillas	-	+	-	-			
Orangs	-	+	+	+	(+)		
Gibbons	-	+	+	+	(+)		
Cercopithecidae (Old World species) ..	-	-	-	-	(+)†	-	
Platyrrhina (New World species)...	-	-	(+)	-	(+)‡	-	
Lemuridae		(+)§	(+)		-	-	

* A part of this table is taken from Wiener (1939).

Symbols. A plus sign (+) indicates the presence of the particular antigen or antigens in the cells of the species; a dash (-) indicates that the character has not definitely been found; parentheses around the plus sign (+) indicate that a similar but not identical component to that in humans is present.

† In 7 species of 4 genera tested.

‡ In only one species (White spider monkey, genus *Ateles*) of 6 species of 4 genera tested.

§ Positive reactions were obtained with bloods of several species of lemurs, indicating the presence of a factor somewhat related to the \bar{A} of humans.

Considering first the characters \bar{A} , \bar{B} and \bar{O} , it has been found (Landsteiner and Miller, 1925a) that the particular antigens, when present alone or together in the blood of the anthropoid apes, are seemingly identical with those of human blood. On this basis it may be concluded that although we have no actual knowledge of the inheritance of the \bar{A} character, for example, in any of these species of apes, it is probable that the gene in each of these species is homologous to the one producing this substance in man.

However, when species of lower monkeys were analyzed (Landsteiner and Miller, 1925b) for the presence of the \bar{A} and \bar{B} antigenic characters of humans, it was noted that comparable blood characters were found only in the New World monkeys (Platyrrhina) and in the Lemuridae. Twelve species (22 individuals in all) taken from 5 genera of the Platyrrhina, and 6 species (8 individuals) of Lemuridae were tested by special reagents for the presence or absence of the \bar{A} and \bar{B} components. A character similar to, but not identical with the \bar{B} anti-

gen of humans was found in the cells of each of these species of the New World, and in the Lemuridae, while no trace of either the \overline{A} or \overline{B} antigens could be detected in the cells of 46 individuals of 18 species of Old World (Cercopithecidae) monkeys. (In the table, parentheses around the "+" sign indicate the presence of an antigen similar to, but not identical with, the particular character.)

Because of the wide-spread occurrence of the Forssman antigen, and a certain chemical relationship which the \overline{A} substance of humans has to it, it is somewhat surprising that there was no definite indication of a character related to the \overline{A} (of humans) in the Old and New World monkeys. As shown in Table I, an identical character appears in chimpanzees, gibbons, gorillas and orangs, and a related but not identical character is found in at least some of the species of lemurs. This might well be taken as evidence of the antiquity of the genes producing both the related and identical substances, the conclusion being that the genes for both \overline{A} and \overline{B} were probably present in a common ancestor of man and the higher apes. Also, since a gene for the \overline{B} -like character is found in the New World monkeys, and not in those of the Old World, and that for \overline{A} found infrequently, if ever, in those families, perhaps the forerunner of the gene for the \overline{B} -substance is the older of the two.

Similar studies (Landsteiner and Wiener, 1937; Wiener, 1938) on the distribution of the \overline{M} and \overline{N} antigens in some of the apes and lower monkeys have shown that no species of those tested contains a character identical with either the \overline{M} or \overline{N} substance of humans. There is, however, a comparable component in the cells of many species, as listed in Table I. This differentiation of the identical and comparable components is possible either by using several immune sera, or by the technic of antibody-absorption. Without the results following agglutinin-absorption it would be assumed that the \overline{MN} ³ com-

³ Tests on twelve chimpanzees have shown that the combination of the \overline{MN} -like components was found in each individual, this combination paralleling the heterozygote (\overline{MN}) which occurs in humans in about 50 per cent.

bination in chimpanzees and the \overline{M} -like characters in oranges, gibbons, and wherever found in other species, were identical with the \overline{M} or \overline{N} characters of humans. The inference from this kind of evidence would be that the same gene was present in all of these species.

The actual results show very clearly that the \overline{M} -like antigen in the different species, although related, is not identical in chemical composition with the \overline{M} character of human cells. Presumably, then, the respective genes producing the \overline{M} -like character in these several species are likewise related to, but not identical with, the gene for the \overline{M} -substance of humans. (The tests made by the workers cited above suggest differences in the M -properties of the cells of the various species of monkeys in which such properties are present. If more critical tests were performed in this respect, it is probable that the \overline{M} -like character would be found not to be identical in more than a few of these species, if in any.) This explanation would apply also to the genes producing the \overline{B} -like characters of the *Platyrrhina* and the lemurs. An alternative explanation would be that the same gene interacting with the different protoplasms of the several species would give a related but different end-product. On the other hand, the fact that the \overline{A} and \overline{B} antigens of humans are identical with characters found in the anthropoids, singly or together, would weaken the alternative proposal.

Let us assume, then, that a gene with an antigenic effect existed in a common ancestor of these different species. It would be reasonable to conclude that, during the evolution of the various species, this gene must have changed in different ways in those different species, so

of the population. One might assume a single gene in the chimpanzee with an effect related to two different antigens in another species (humans). This, however, seems somewhat improbable. It would appear that, unless these characters are found separately in other chimpanzees, there is in this species an instance of genes with contrasting effects always occurring together, presumably being on the same chromosome, whereas the genes in humans related to each of these are alleles. Possibly the explanation for the bar-eye effect in *Drosophila* would apply here.

that its effects, while similar, are not identical. We can only speculate as to whether this particular gene in each of these species is an allelic relation to that of the humans. The decreasing degree of similarity noted (*loc. cit.*) between some of the species for both the \overline{B} -like and \overline{M} -like antigens would suggest also that there are different degrees of relationship in the genes involved, among themselves, and to those in man.

From this and other evidence, we may conclude that there probably are three categories of genes which make for resemblances and differences between related species. In the first class there are those genes of the two species which are homologous, and which produce the same effects. In the next class are genes which are related but not identical, and which produce similar but not identical effects. (Naturally, if the relationship of this class of genes, one to the other, was that of one allele to another, although found in different species, this category would be superfluous.) Finally, in the third category there are those genes which are not alike and which make for the differences that exist between one species and another. Presumably, we would expect that the relative relationship between species would depend upon the proportion of their respective genes that were shared, in contrast with those that were specific for either species.

If within different species there were many genes with effects upon the antigens of the red blood cells, it would be anticipated that an immunological distinction between the cells of any two species could be readily accomplished. Such a comparison is shown in Fig. 1 for the two species of doves, Pearlneck (*Streptopelia chinensis*) and Ring Dove (*St. risoria*). It will be noted that each species shares a part of its cellular pattern with the other and, in addition, each has a part which is peculiar to itself. (The proportions in the diagram have of necessity been set arbitrarily.) Further, the species hybrid contains all of the properties common to both parents, nearly all of the species specific properties of each, and one or more characters not found in either parent.

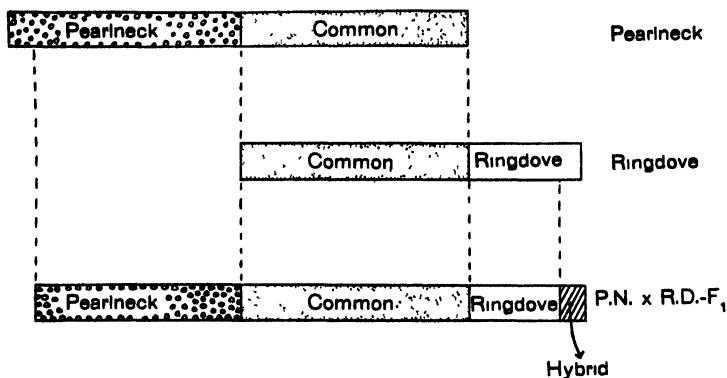


FIG. 1. A diagram of the comparison of the antigenic composition of the cells of Pearlnecks, Ring doves and the F_1 hybrids.

These characters peculiar to the hybrid are of interest because either the same or related substances are normally found in a few other species. Presumably, then, certain of the genes, which by their interaction in the hybrid produce the hybrid components, are a part of the normal genic complex of other species. The production of these "hybrid components" does not seem to be an attribute of all species hybrids. In only two of the seven different species hybrids in doves tested to date has there been definite evidence of a "new" or "hybrid" substance. Other than these components, the relationship of each of the different species hybrids to the common and specific components of the two parental species is approximately as pictured for the hybrid between Pearlneck and Ring Dove.

Following continued backcrosses to the Ring Dove, a separation of the specific Pearlneck components has been observed (Irwin, 1939). Ten distinct major Pearlneck characters have been isolated so that representatives of any single character, when mated to Ring Doves, produce offspring of which approximately half contain the particular character and half do not. This evidence, combined with that from other sources, practically establishes the veracity of the statement that the antigens of the red blood cells are gene-determined. Comparisons of

the cellular antigens of different species are then somewhat more than an indirect way of measuring the gross gene relationships of the species.

Utilizing as testers the unit-antigens of Pearlneck, it has been found that Pearlneck possesses in common with other related species, and in varying combinations, certain of the antigens not shared with Ring Dove. What then from these studies as a whole can we say concerning the genic complex of a single species in relation to a number of other species? Does each species have particular genes which set it apart from all others? Or is a species simply a different combination of a given number of genes, all of which might be present if the genes of two or more other species were combined?

An absolute answer to these alternative questions can not be given at present. However, rather exhaustive tests on the antigenic composition of two species, Pearlneck and *Columba guinea*, and less exhaustive tests on several other species of pigeons and doves, entirely substantiate the proposal that each species possesses certain genes with antigenic effects not found at all in other species. The remainder of the antigenic pattern of the species is shared, in many different combinations, with other related species, presenting a most intricate pattern of species relationships.

An interesting point has been noted as the result of comparisons of the cellular substances of the different species of pigeons (*Columba*) carried out in our laboratory. We have made extensive tests of the inter-relationships of five species of Old World and six species of the New World pigeons,⁴ which in summary suggest a correlation of relationship with habitat in the two hemispheres.

Briefly, as may be noted in Fig. 2, there appear to be antigens which are common to species of both the Old and

⁴ The species of pigeons of the Old World are *Columba guinea*, *C. livia domestica*, *C. janthina*, *C. oenas* and *C. palumbus*. Those of the New World, *C. fasciata*, *C. flavirostris*, *C. leucocephala*, *C. maculosa*, *C. picazuro* and *C. rufina*.

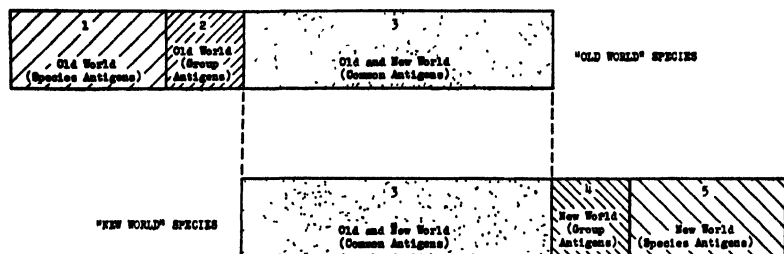


FIG. 2. Interrelationships of antigens of Old and New World species of the genus *Columba*.

1. Antigens of Old World species of *Columba*, including those characteristic of each species.
2. Antigens of Old World species of *Columba*, contained in many if not in all Old World species.
3. Antigens shared by both Old and New World species of *Columba*.
4. Antigens of New World species of *Columba*, contained in many if not in all New World species.
5. Antigens of New World species of *Columba*, including those characteristic of each species.

the New World. Further, within the species of the Old World there are other antigens which set these species apart as a group from any of those of the New World. These components are shared by many, if not by all, of the Old World species. And then there are other antigens which set each species apart from every other species of the Old World. Likewise, the species of the New World have a group of cellular characters which are not found in those of the Old World, and which are present in most, if not in all, of the species of the New World. Also, each species has its own distinctive antigens, as stated above.

As a corollary, we may say that by these tests, and for these species, we have found that any species of pigeon of the New World is more similar to any other species of the New World than it is to any Old World species. Likewise, any species of the Old World is more like any other pigeon of the Old World than is any species of the New World.

A very few exceptions to these general relationships have been observed, and more may become apparent when and if representatives of other species of pigeons

are available for inclusion in these tests. However, these exceptions do not, in our opinion, detract from the authenticity of the more or less sharp differentiation of the species from the Old and New World, respectively.

At the present time, it seems that these analytical procedures may eventually enable us to fit the many species of pigeons and doves into a phylogenetic pattern, to which it is our hope that a genetic explanation can be applied.

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BREEDING STRUCTURE OF POPULATIONS IN RELATION TO SPECIATION¹

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INTRODUCTION

THE problem of speciation involves both the processes by which populations split into non-interbreeding groups and those by which single populations change their characteristics in time, thus leading to divergence of previously isolated groups.

The first step in applying genetics to the problem is undoubtedly the discovery of the actual nature of the genetic differences among allied subspecies, species and genera in a large number of representative cases. Differences which tend to prevent cross-breeding are obviously especially likely to throw light on the process of speciation, but all differences are important.

Our information here is still very fragmentary. We know enough, however, to be able to say that there is no one rule either with respect to cross-sterility or to other characters. In some cases the most significant differences seem to be in chromosome number and organization. At the other extreme are groups of species among which gross chromosome differences and even major Mendelian differences are lacking, both cross-sterility and character differentiation depending on a multiplicity of minor gene effects. In general, there are differences at all levels (*cf.* Dobzhansky, 1937).

But even if we had a complete account of the genetic differences within a group of allied species, we would not necessarily have much understanding of the process by which the situation had been arrived at. A single mutation is not a new species, except perhaps in the case of polyploidy. The symmetry of the Mendelian mechanism

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is such that any gene or chromosomal type tends to remain at the same frequency in a population except as this frequency is changed either by some steady evolutionary pressure (such as that due to *recurrent* mutation, to various kinds of selection, to immigration and to differential emigration) or by the accidents of sampling, if the number of individuals is small. The elementary evolutionary process, from this view-point, is change of gene frequency.

It is to be expected that the nature of the process will be found to be affected by what I have called the breeding structure of the species, and it is this aspect of the matter that I wish to discuss here. Such a discussion involves at least three steps. First, there is the observational problem of determining what the breeding structures of representative species actually are. Naturalists are only beginning to collect the detailed information which turns out to be necessary, but that which we have indicates situations of great complexity. The second step is that of constructing a mathematical model which represents adequately the essential features of the actual situation while disregarding all unimportant complications. The third step is the determination of the evolutionary implications of a given breeding structure in relation to mutation and selection. As difficult problems of description and mathematical formulation are also involved in the cases of mutation and selection pressures, the whole problem is exceedingly complex. I can only discuss the implications of certain very simple models of breeding structure, chosen partly because they appear to correspond to situations which one might expect to find in nature, but partly also because of mathematical convenience.

EVOLUTION UNDER PANMIXIA

The simplest situation, under biparental reproduction, is that of a large population, breeding wholly at random (panmixia). If sufficiently large, variability due to accidents of sampling is negligible. Each gene frequency

shifts steadily under the pressures of selection and recurrent mutation. Mathematical formulations of these pressures have been given. Letting q be the frequency of a given gene, $(1 - q)$ that of its alleles, u and v the mutation rates respectively from and to the gene in question and \bar{W} the mean selective value of all possible genotypes, weighted by their frequencies, the change in gene frequency in a generation is given by the following formula (Wright, 1937):

$$\Delta q = v(1 - q) - uq + \frac{q(1 - q)\delta\bar{W}}{2\bar{W}\delta q}$$

For a gene which causes the same difference from its allele in all combinations and which lacks dominance, the term for selection pressure reduces approximately to $sq(1 - q)$, where s is the selective advantage over the allele.

The numbers of generations necessary for any given shift in gene frequency, under various hypotheses, have been presented by Haldane (1932 and earlier). This sort of process has been taken as typical of evolutionary change by R. A. Fisher (1930), who has compared its unswerving regularity to that of increase in entropy in a physical system.

If, however, conditions are constant, this process comes to an end at an equilibrium point at which opposing pressures balance each other ($\Delta q = 0$). At this point there is stability of the species type in spite of continual occurrence of mutations, an extensive field of variability and continuous action of selection. On the other hand, conditions never are wholly constant. It is possible that evolution, in each series of alleles, may consist of an unswerving pursuit of an equilibrium point, which is itself continually on the move because of changing conditions.

The postulate that variations in gene frequency, due to accidents of sampling, are negligible calls for some comment. The variance in one generation is $\sigma^2_{\Delta q} = \frac{q(1 - q)}{2N}$ in a diploid population of effective size N . This is cumu-

lative and may cause wide divergence from equilibrium if the population is not too large. The systematic evolutionary pressures directed toward equilibrium and this sampling variance determine between them a certain distribution of values of the gene frequency instead of a single equilibrium point. The general formula can be written as follows (Wright, 1937):

$$\varphi(q) = (C/\sigma^2_{\Delta q}) e^{2 \int (\Delta q / \sigma^2_{\Delta q}) dq}$$

For the values of Δq and $\sigma^2_{\Delta q}$ given above this reduces to

$$\varphi(q) = C \bar{W}^{2N} q^{4Ns-1} (1-q)^{4Nu-1}$$

In the special case of no factor interaction and no dominance, the term \bar{W}^{2N} becomes approximately e^{4Ns} . There is a marked tendency toward chance fixation of one allele or another if $4Ns$, $4Nv$ and $4Nu$ are all less than 1 while such variability is negligible if these quantities are large (*e.g.*, as large as 100).

The possible evolutionary significance of these random variations in gene frequency in a panmictic population has been considered elsewhere (Wright, 1931, 1932) and will not be discussed further here.

Mating never is wholly at random. It is important to determine whether departures from panmixia have significant effects on the evolutionary process and if so whether these consist merely in impeding the pursuit of equilibrium or whether they may not bring about progress of a different sort.

One limitation on the effectiveness of selection in a panmictic population is that it can apply only to the *net effects* in each series of alleles. It is really the organism as a whole that is well or ill adapted. A really effective selection pressure should relate to genotypes not genes. But in a panmictic population, combinations are formed in one generation only to be broken up in the next.

If a selective value (W) is assigned to every one of the practically infinite number of possible combinations of genes of all loci, the array of such values forms a surface in a space of at least as many dimensions as there are loci, more if there are multiple alleles. Because of non-

additive factor interactions, this surface in general has innumerable distinct peaks (*i.e.*, harmonious combinations) each surrounded by numerous closely related but slightly less adaptive combinations and separated from the others by valleys. Selection according to net effect can only carry the species up the gradient to the nearest peak but will not permit it to find its way across a valley to a higher peak. Evolution would have a richer field of possibilities under a breeding system that permitted exploration of neighboring regions in the surface of adaptive values, even at some expense in momentary adaptation.

A somewhat similar situation holds within systems of *multiple alleles* (*cf.* Timoféeff-Ressovsky, 1932). There is presumably a limit to the number of alleles that can arise from a given type gene by a single act of mutation. But each of these mutations presumably can give rise to mutations at two steps removed from the original type gene and so on in an indefinitely extended network. If there is approximate fixation of one allele (to be expected in general under panmixia), only those mutations that are at one or two removes have any appreciable chance of occurrence. There will be continual recurrence of the same mutations without real novelty. A breeding system that tolerates a continually shifting array of multiple alleles in each series in portions of the population, gives the opportunity for a trying out of wholly novel mutations which occasionally may be of great value. The question then is whether there are breeding structures that permit trial and error both within each system of multiple alleles, and within the field of gene combinations, in such a way as to give a richer field of possibilities than under the univalent determinative process in a panmictic population.

EVOLUTION UNDER UNIPARENTAL REPRODUCTION

At the opposite extreme from the system of random mating is that in which there is uniparental reproduction.

Under vegetative multiplication, or under diploid parthenogenesis, each individual produces a clone in which all individuals are of exactly the same genotype, except for occasional mutations. Continued self fertilization also leads to the production of groups of essentially identical individuals.

Suppose that a highly variable panmictic population suddenly shifts to uniparental reproduction. Selection then would be between genotypes. Those combinations that are most adaptive would increase, including perhaps rare types that would have been broken up and lost under panmixia. The less adaptive combinations would soon be displaced. Selection would be exceedingly effective until only one clone was left in each ecological niche. But at this point evolution would come to an end, except for the exceedingly rare occurrence of favorable mutations.

It is obvious that a certain combination of the preceding systems should be much more effective than either by itself (*cf.* Wright, 1931). Prevailing uniparental reproduction, with occasional crossing would permit an effective selection by genotypes to operate in a continually restored field of variability. This combination is of course one that has been used most effectively by plant breeders. It is found in many plants and animals in nature and has presumably been an important factor in their evolution.

The demonstration of the evolutionary advantages of an alternation of periods of uniparental reproduction with cross-breeding may seem to prove too much, since it is not usual in those groups that are usually considered to have evolved the most, the higher arthropods and vertebrates. Perhaps, however, there are other systems which also bring about differentiation of types and thus a basis for selection based on type rather than mere net gene effect, and which have more stability than arrays of clones.

EVOLUTION IN SUBDIVIDED POPULATIONS

A breeding structure that happens to be very conveni-

ent from the mathematical standpoint is one in which the species is subdivided into numerous small local populations which largely breed within themselves but receive a small proportion of their population in each generation from migrants which can be treated as random samples from the species as a whole. The basis for the partial isolation may be geographical, or ecological or temporal (breeding season). In the latter two cases an adaptive difference is postulated. We are not here considering the origin of this but rather its consequences on other characters.

Whatever the mechanism of isolation, its evolutionary significance can be evaluated in terms of the effective size of population (N) of the isolated group, the effective rate (m) of exchange of individuals between the group (gene frequency q) and the species as a whole (gene frequency q_t) and the local selection coefficient. It will be convenient here to write s for the net selection coefficient and to ignore mutation pressure (Wright, 1931).

$$\Delta q = sq(1-q) - m(q - q_t)$$

If s in a local population is much larger than m , we have approximately

$$\hat{q} = 1 - \frac{1}{s} [m(1 - q_t)] \quad \text{if } s \text{ is positive}$$

$$\hat{q} = \frac{mq_t}{(-s)} \quad \text{if } s \text{ is negative}$$

If the values of s among local populations show differences greater than m , there will be marked adaptive differentiation of such populations. There is an approach toward fixation of the locally favored gene largely irrespective of the frequency in the species as a whole.

The importance of isolation in evolution seems to have been urged first by M. Wagner as permitting divergent evolution under the control of different environments. Wagner thought of environment as directly guiding the course of evolutionary change, when its effects were not swamped by those of cross-breeding. A similar view has been held by many others since his time who have considered such orderly clines among geographical races as

those described by the laws of Bergmann, Gloger, and Allen. While direct control over mutation is not in line with present knowledge of genetics, indirect control through differential selection seems probable enough in these cases (*cf.* Dobzhansky, 1937; Huxley, 1939).

Davenport (1903) and Goldschmidt (1934) have stressed the likelihood of the spreading of the range of species by the diffusion of preadaptive mutations into territories in which they are isolated from the first by the inability of the typical members of the species to live. Goldschmidt has interpreted the major differences among races of *Lymantria dispar* in this way. He finds these differences primarily in such physiological characters as developmental rate, length of diapause, etc. Mathematically, this would be a special case of the foregoing scheme.

Differential selection has been considered so far as a factor making only for divergence of groups within the species and thus tending toward splitting of the latter. There is a possibility, however, that it may be a factor making for progressive evolution of the species as a unit. Particular local populations may, by a tortuous route, arrive at adaptations that turn out to have general, instead of merely local, value and which thus may tend to displace all other local strains by *intergroup* selection (excess emigration). In terms of our multidimensional surface of adaptive values, a particular substrain may be guided from one peak to another by a circuitous route around a valley which would probably not have been found except by such a trial and error mechanism. As different alleles may approach fixation in different populations, mutations at two or more removes from the original type have more opportunity for occurrence than if the population were homogeneous. Thus there may be trial and error within series of alleles as well as between gene combinations.

Let us now turn to the case in which the local selection coefficient is smaller instead of larger than m . The local equilibrium frequency (\hat{q}) is approximately as follows.

$$\hat{q} = q_t + \frac{sq_t}{m}(1 - q_t)$$

The values in different local populations in which s is smaller than m are clustered closely about the mean gene frequency, q_t . Selection causes no important differentiation. There may however be variability of each local population due to accidents of sampling if N is small and, consequently, much non-adaptive differentiation among such populations at any given moment.

$$\varphi(q) = Ce^{Ns(q - q_t)^{-1} (1 - q)^{Nm(1 - q_t) - 1}}$$

Figure 1 shows the form of the distribution for various values of Nm , taking $q_t = \frac{1}{2}$ and assuming no selection ($s = 0$). The variance in this case is as follows.

$$\sigma^2 q = \frac{q_t(1 - q_t)}{4Nm + 1}$$

The distribution of gene frequencies is U shaped, implying random drifting from fixation in one phase to another if m is less than $\frac{1}{4Nq_t}$ and $\frac{1}{4N(1 - q_t)}$. This again would permit trial within each series of alleles, and also between gene combinations.

The latter at least would be important even with larger values of m relative to $\frac{1}{4}N$. With $Nm = 5$, the standard deviation of values of q is 22 per cent. of its limiting value $\sqrt{q(1 - q)}$. Such variability tends to become unimportant however if Nm is much larger.

Gulick seems to have been the first to point out the possible significance of isolation in bringing about a non-adaptive differentiation of local races. He has been followed by others, notably recently by Kinsey in his studies of the gall wasps of the genus *Cynips* (1929, 1936). A study of eleven isolated mountain forests in the Death Valley region by Dobzhansky and Queal (1938) showed a close approach to random mating with no appreciable selection within localities. Between localities on the other hand, frequencies ranged from 51 per cent. to 88 per cent., 2 per cent. to 20 per cent., 8 per cent. to 39 per cent. with standard deviations which can be accounted for by an effective value of Nm of about 5.1. The much greater standard deviation for the range of *D. pseudo-obscura* as a whole shows that this differentiation is cumulative with distance.

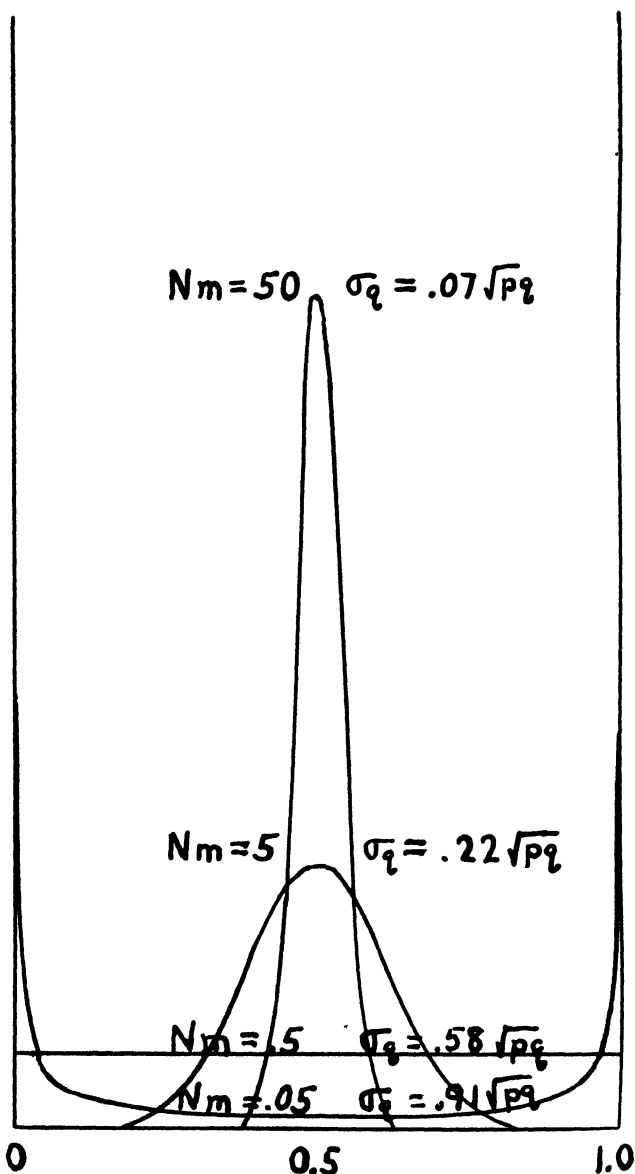


FIG. 1. The distribution of gene frequencies for various values of N_m , taking $q_1 = \frac{1}{2}$ and assuming no selection. The symbol p is used for $1-q$.

EFFECTIVE SIZE OF POPULATION

There appears to be the difficulty here that the number of individuals in such a form as *Drosophila* is so enor-

mous that it is difficult to conceive of a limitation in numbers as having any appreciable sampling effects. However, the effective N may be very much smaller than the apparent N (Wright, 1938).

If the number of the two sexes (N_m males, N_f females) is unequal, it can be shown that effective $N = \frac{4N_mN_f}{N_m + N_f}$. With unequal numbers, the effective size of population depends more on the smaller number than on the larger number. Thus with N_m males but an indefinitely large number of females, $N = 4N_m$.

Again, different parents may produce widely different numbers of young. If σ^2_κ is the variance in number of gametes contributed by individuals to the following generation in a population (N_0) that is maintaining the same numbers ($\bar{\kappa} = 2$), $\sigma^2_\kappa = \frac{\sum (\kappa - 2)^2}{N_0}$

$$N = \frac{4N_0 - 2}{2 + \sigma^2_\kappa}$$

The effective size of population is twice as great as the apparent in the highly artificial case in which each parent contributes just two gametes. Effective and apparent size of population are the same if the number of gametes contributed by different parents vary at random (Poisson distribution). If, as would often be the case, most of the offspring come from a small percentage of the mature individuals of the parental generation, the effective size would be much less than the apparent size.

A population may vary tremendously in numbers from generation to generation. If there is a regular cycle of a few generations ($N_1, N_2 \dots N_n$) an approximately equivalent constant population number can be found.

$$N = \frac{n}{\sum_{x=1}^n [1/N_x]}$$

This is controlled much more by the smaller than by the larger numbers. Thus if the breeding population in an isolated region increases ten-fold in each of six generations during the summer (N_0 to $10^6 N_0$) but falls at the

end of winter to the same value, N_0 , the effective size of population ($N = 6.3N_0$) is relatively small.

In such a cycle, certain individuals in favorable locations are likely to start reproduction earlier than others, perhaps getting a start of a whole generation. In a rapidly breeding form, these few individuals would contribute overwhelmingly more than the average to all later generations. Thus, by a combination of the two preceding principles, the effective size of population may be very small indeed.

The possible evolutionary significance of periodic reduction in the size of natural populations has been discussed by a number of authors. Elton (1934) especially has maintained that chance deviations in the characteristics of survivors at the time of least numbers may have important effects of this sort.

An important case arises where local populations are liable to frequent extinction, with restoration from the progeny of a few stray immigrants. In such regions the line of continuity of large populations may have passed repeatedly through extremely small numbers even though the species has at all times included countless millions of individuals in its range as a whole (*cf.* Fig. 2).

Such mutations as reciprocal translocations that are very strongly selected against until half fixed seem to require some such mechanism to become established. There is an exceedingly deep valley in the surface \bar{W} representing the mean adaptive value in populations with given frequencies of old and new chromosomes, and the term \bar{W}^{2N} in the formula for the joint chromosome frequencies is so small, where N consists of more than some half-dozen individuals that fixation is virtually impossible. Yet translocations have been noted between *Drosophila* species (*e.g.*, *D. pseudoobscura* and *D. miranda*, Dobzhansky and Tan, 1936) although they are far less common than inversions. The difficulty referred to here does not, of course, apply in species that reproduce vegetatively or by self-fertilization.

We have discussed various considerations that make

effective N much smaller than at first apparent. The effective amount of cross-breeding may also be much less than the actual amount of migration seems to imply. Most of the immigrants are likely to come from neighboring groups, differing less from the receiving population in gene frequency than would a random sample from the

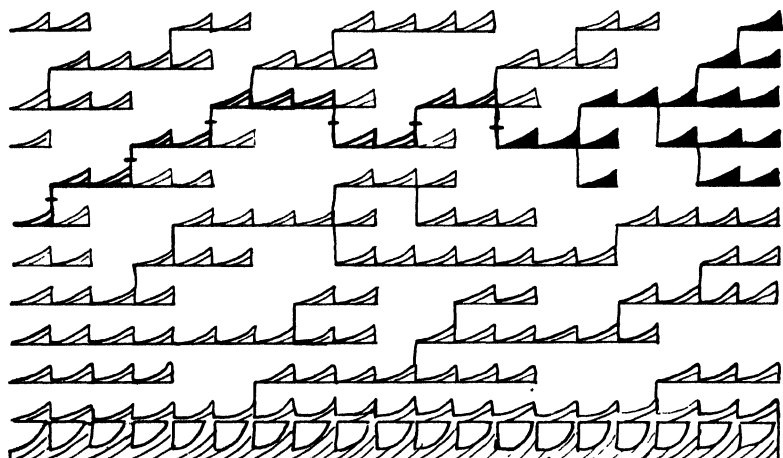


FIG. 2. Diagram of breeding structure in a species in which the populations in certain regions are liable to frequent extinction with reestablishment by rare migrants. Different territories are distinguished vertically. Generations proceed from left to right horizontally. The heavily shaded group represents a large population the entire ancestry of which has passed through small groups of migrants six times in the period shown.

species. If there is a correlation, r , between immigrants and receiving group, the m of the formula must be replaced by $m(1-r)$ if m is to continue to be the actual amount of replacement by immigration.

In the case of *Drosophila pseudoobscura*, it has been noted that Dobzhansky and Queal (1938) found variability in gene frequency among mountain forests of the Death Valley region which implied an effective value of Nm of about 5. For the species as a whole, variability is such that effective Nm must be only about one tenth as large as this (0.5).

ISOLATION BY DISTANCE

This last case leads to another model of breeding struc-

ture which may be of considerable importance (Wright, 1938). Suppose that a population is distributed uniformly over a large territory but that the parents of any given individual are drawn from a small surrounding region (average distance D , effective population N). How much local differentiation is possible merely from accidents of sampling? Obviously the grandparents were drawn from a larger territory (average distance $\sqrt{2} D$, effective population $2N$). The ancestors of generation n came from an average distance $\sqrt{n} D$ and from a population of average size nN . It is assumed that the variance of the ancestral range, either in latitude or in longitude, increases directly with the number of generations of ancestry.

Fig. 3 shows how the standard deviation of gene frequencies for unit territories of various effective sizes increases with distance. If $c_q = .577\sqrt{q_t(1-q_t)}$ and $q_t = 1 - q_i = \frac{1}{2}$ all values of gene frequency are equally numerous ($\phi(q) = 1$). Any larger value implies a tendency toward fixation of one or the other allele in different local populations.

If the parents are drawn from local populations of effective size greater than 1,000, the situation differs little from panmixia even over enormous areas. There is considerable fluctuating local differentiation of unit territories where their effective size is of the order of 100, but not much differentiation of large regions unless effective N is much less.

Kinsey's (1929) description of the gall wasp, *Cynips pezomachoides erinacei*, conforms fairly well to the above model for the case of moderately large N . This subspecies ranges over some 500,000 square miles in north-eastern United States. Both the insects and their galls may differ markedly and consistently in collections taken from different trees or small groves at short distances apart, but the same variability is found throughout the range. There is little regional differentiation in this enormous territory, although at still greater distances

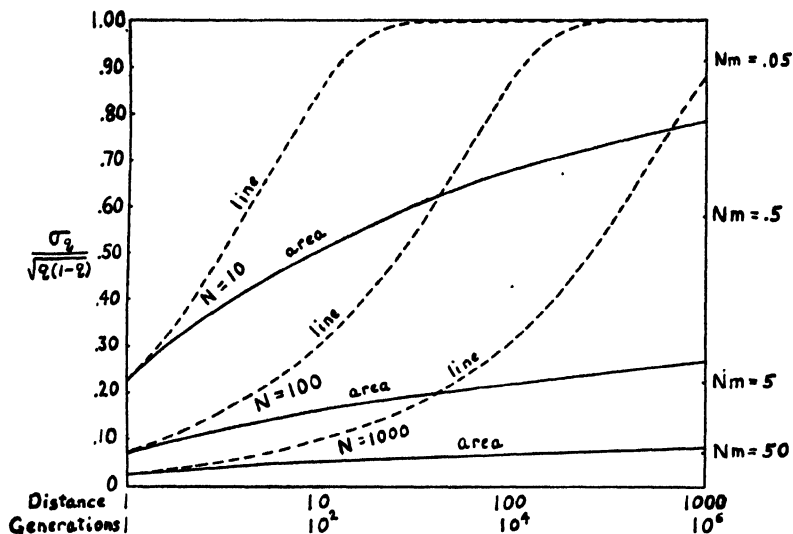


FIG. 3. The standard deviation of the mean gene frequencies of unit random breeding territories ($N = 10$; $N = 100$; $N = 1000$), in relation to mean distance. The case in which the population is distributed uniformly over an area is represented in solid lines, that in which it is distributed along one dimension by broken lines.

the species complex *C. pezomachoides* is subdivided into eight intergrading species.

In a species whose range is essentially one-dimensional (shore line, river, etc.) the ancestors of generation n come from an average distance of $\sqrt{n} D$ as before, but the effective size of population is $\sqrt{n} N$ instead of nN . Differentiation increases much more rapidly with distance than in the preceding case. This principle was suggested in qualitative terms by Thompson (1931) in his study of differentiation within species of river fish in relation to water distance. In weak swimmers (e.g., Johnny Darters) there was marked increase in average difference in number of fin rays with increased distance in spite of a continuous distribution. The differentiation with distance was not as rapid, however, as that in several species with discontinuous distribution (restricted to the smallest stream). On the other hand the strong swimmers of the rivers showed little or no differentiation throughout their entire ranges.

Such uniformity in breeding structure as implied above is not likely to be closely approximated in nature. Even where there is apparent continuity of a population, it is likely that conditions vary from place to place in such a way that there is excess multiplication at certain centers separated by regions in which the species would be unable to maintain itself permanently were it not for immigration (as in the breeding structure of Figure 2). Moreover, even with complete uniformity of conditions, local differentiation should result in the accidental attainment of more adaptive complexes in some regions than in others. As before, incipient nonadaptive differentiation may lead to a more important adaptive differentiation. The centers in which population is increasing most rapidly will become increasingly isolated from each other by the mere fact that they are centers of emigration.

A process of this sort has been postulated by Sumner (1932) in the case of subspecies of *Peromyscus*. Within subspecies, he found statistical differentiation of most local populations which may well have been of the type due merely to distance. But at the subspecies boundaries there was typically a zone of relatively rapid change. These boundaries were not necessarily along natural barriers to migration. Sumner compared them with the distributions which would result "if a collection of spherical rubber bags were placed in rigid containers and then strongly but unequally inflated."

The breeding structure of natural populations thus is likely to be intermediate between the model of subdivision into partially isolated territories and that of local inbreeding in a continuous population. In so far as it is continuous, it is likely to be intermediate between area continuity and linear continuity.

Summing up, we have attempted to show that the breeding structure of populations has a number of important consequences with respect to speciation. Partial isolation of local populations, even if merely by distance is important, not only as a possible precursor of splitting of the species, but also as leading to more rapid evolu-

tionary change of the population as a single system and thus more rapid differentiation from other populations from which it is completely isolated. Local differentiation within a species, based either on the nonadaptive inbreeding effect or on local conditions of selection or both, permits trial and error both within series of multiple alleles and between gene combinations and thus a more effective process of selection than possible in a purely panmictic population.

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SPECIATION PHENOMENA IN BIRDS¹

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EVOLUTION is a very complicated and many-sided process. Every single branch of biology contributes its share of new ideas and new evidence, but no single discipline can hope to find all the answers or is justified to make sweeping generalizations that are based only on the evidence of its particular restricted field. This is true for cytology and genetics, for ecology and biogeography, for paleontology and taxonomy. All these branches must cooperate and the worker in each field must try to apply an approach to the problems of evolution that is particularly suitable to the methods of his special field. It is obvious that the taxonomist will not find out very much about the origin of new genetic characters nor about their transmission from one generation to the next. On the other hand, the taxonomist will be able to give answers to certain questions which are not attainable by the geneticist since speciation is not a purely genetic process. To-day I shall try to discuss, as taxonomist and zoogeographer, some such questions relating to speciation in birds.

SPECIATION PHENOMENA IN BIRDS

Birds are a peculiar field for the study of speciation phenomena; in some ways they are particularly well suited for such studies and in other ways they are quite unsuitable. They are unsuitable, because the experimental genetic work is difficult and yields only meager results. The reason for this is that most wild birds are very difficult to breed; secondly, the sequence of generations is very slow (usually only one per year), and the number of offspring small; the number of chromosomes

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is very large and nearly every character that has been carefully examined is determined by a whole bevy of modifying genes.

To offset these disadvantages there are a number of unusual advantages. The principal one is that birds are better known taxonomically and biologically than any other group of animals. It is estimated that less than 100 species of birds remain undiscovered in the entire world, among an estimated total of 8,500 species, that is, about 1 per cent. (Mayr, 1935). In the more thoroughly investigated regions of the world (N. America, Europe) not a single new good species has been discovered in more than forty years. About three good species of birds are discovered annually in the entire world by numerous collecting parties and several score of working taxonomists. This amounts to an annual addition of $1/30$ per cent. to the known number of species. The total number of valid subspecies and geographical races which are being described annually is about 200, or $\frac{1}{3}$ per cent. of the known 26,000 species or subspecies. Other groups of animals are very much less known. In the Cynipidae (gall wasps), for example, one single collector (Kinsey) added during the course of two seasons of field work no less than 36 species to the known 50 species of one genus, an addition of 72 per cent.

But in birds not only most of the forms are known, but also their exact geographical distribution and their variation. In fact, most taxonomic work on birds during the last fifty years has been devoted to the subdivisions of the species, the geographical races or subspecies. The methods and aims of this field closely approach those of the student of the genetics of populations, and it is no wonder that a close understanding between taxonomist and geneticist has developed in recent years.

SPECIES DEFINITION

"Speciation or species formation is the process by which new species develop." This definition leads in-

evitably to the next question: "What is a species?" This question has bothered taxonomists and biologists alike ever since biologists, especially Linnaeus, firmly established the species concept, but the confusion has grown steadily with the constant refinement of taxonomic technique and with the advent of evolutionary thought. Unfortunately, we can not dispose of the question simply by dodging the definition as irrelevant, because, for example, a good many controversies are primarily due to the different species concepts held by the opponents (*cf.* Goldschmidt, 1937).

The difficulties and possible objections against a species definition can best be illustrated by analyzing and criticizing a number of the definitions given by well-known taxonomists or geneticists.

To Linnaeus the species was a unit that could be defined on a morphological basis. Consequently, he described in numerous cases males and females, young and adult, as different species, because they had well-definable morphological characters. Furthermore, the species was for him, in general, the lowest systematic unit. I do not have the time to trace the historical changes of the species concept. It has been done by Stresemann (1936) so far as ornithology is concerned. There is no doubt, however, that the species concept in most taxonomic groups has undergone an almost revolutionary change during the last thirty to forty years. Painstaking taxonomic work, particularly by ornithologists, lepidopterologists and conchologists, has shown that gliding intergradation connects most geographically representative species, so that they had to be reduced to subspecies. In Darwin's day the discovery of such transitions was hailed as proof of the change of one species into another. Nowadays we know that it is the normal condition and that there are relatively few "good" species that are not actually composed of groups of "subspecies." A few taxonomists, especially entomologists, are left who still insist in calling species the lowest

systematic categories. The best-known advocate of this is Kinsey (1937b) whose point of view is as follows:

Confusion will be avoided if we call the basic taxonomic unit the species. It is the unit beneath which there are in nature no subdivisions, which maintain themselves for any length of time or over any large area. The unit is variously known among taxonomists as the species, subspecies, variety, *Rasse* or geographic race. It is the unit directly involved in the question of the origin of species, and the entity most often indicated by non-taxonomists when they refer to species. Systematists often introduce confusion into evolutionary discussions by applying the term to some category above the basic unit.

Goldschmidt (1937) has already voiced some objections to these claims, but I might add a few remarks. It is not true that "there are in nature no subdivisions" below the species [of Kinsey], "which maintain themselves for any length of time." Actually there are all degrees of distinctness between "the effective breeding population" within a continuous array of populations and the subspecies which is completely isolated by geographical barriers. Recent genetic work (Dobzhansky, *et. al.*) as well as Kinsey's own taxonomic work shows this quite clearly. Furthermore, the lowest category is not "the entity most often indicated by non-taxonomists when they refer to species." When the layman or non-taxonomic biologist speaks of "the song sparrow," he is not concerned with the numerous subspecies of this species, as for example the Atlantic, the Eastern or the Mississippian race. He means the total sum of all these races, or else, the particular local race wherever he meets it. Neither do we call the human races species, although they are the basic taxonomic units of *Homo sapiens*.

Emerson's definition (1938), I am afraid, is equally vulnerable. He says: "A species is a genetically distinctive, reproductively isolated, natural population." To begin with, every "natural population," no matter what its rank is, that has yet been genetically analyzed, has turned out to be "genetically distinctive." Every individual (unless it be an identical twin) has an overwhelming chance to be genetically distinct from every other member of his own species due to the almost infi-

nite possible combinations of alleles, and therefore the genetic distinctness is of no great value in a species definition. More difficulties are provided by the clause "reproductively isolated." The chief trouble with this is that it can rarely be tested. In the tropics, for example, birds of many species are exceedingly sedentary. If two populations of one species occupy two separated mountain-tops or islands, they are unquestionably "reproductively isolated," as effectively as is conceivable. Still some major climatic change could bring them together again and they would unquestionably freely hybridize on meeting each other. Artificial breeding experiments might not solve anything in such a case, because in captivity related species are known to hybridize which fail to do so in nature. This is known for *Cepaea hortensis* and *nemoralis* which in nature commonly live side by side with hybrids being exceedingly rare, and also for many species of birds.

Timoféeff (1940) overcomes most of the difficulties of other definitions but is equally weak against the last of the objections just stated. He writes:

A species is a group of individuals that are morphologically and physiologically similar (although comprising a number of groups of the lowest taxonomic category) which has reached an almost complete biological isolation from similar neighbouring groups of individuals inhabiting the same or adjacent territories. Under biological isolation we understand the impossibility or nonoccurrence of normal hybridisation under natural conditions.

Most geographically isolated forms would be species under this definition. Dobzhansky (1937) wants to overcome this difficulty (which he realizes very clearly) when he defines species as: "that stage of the evolutionary process at which the once actually or potentially interbreeding array of forms becomes segregated in two or more separate arrays which are physiologically incapable of interbreeding." This definition is an excellent description of the process of speciation, but it defines what a group of species is (*i.e.*, two or more separate arrays), not what *one* species is. I do not share Emerson's (*l.c.*) objections (production of fertile offspring between re-

lated species), because a modern ornithologist does not consider as full species two neighboring "species" which hybridize freely in their zone of contact. My principal objection against Dobzhansky's definition is that he leaves difficult situations of practical importance undecided. For example, how does his definition deal with those cases where a species (in the widest sense, not in Dobzhansky's) consist of a long geographical chain of different populations or subspecies, of which each one intergrades with its neighbors and is not separated by any physiological isolating mechanisms, but where the final members of the chain are completely sterile when brought together. There are several such cases known from beetles (*Epilachna*, *Carabus*) and a number of less drastic ones from other groups. Second, how can it be practically tested that two forms are "physiologically incapable of interbreeding"? There are probably more than a million species of animals in existence and in less than 1/10 of 1 per cent. have crosses with one another been attempted. Should we wait until the other 99 9/10 per cent. have been crossed before these species can be fully established?

Before I try to give my own species definition we might analyze very briefly what criteria are generally used to define species.

(a) *Morphological characters*: Descriptive characters, such as structure, proportions, color patterns, etc., are the conventional means used to define a species. It is therefore only natural if even modern species definitions state that a species is composed of "groups of individuals with similar morphological characters." However, geographical forms differ in certain families (*Phasianidae*, *Paradisaeidae*, *Trochilidae*) by "stronger" morphological characters than good species in others (*Apodidae*, *Tyrannidae*, *Zosteropidae*). Perfectly good species are often remarkably similar, I only need to remind you of *Drosophila pseudoobscura* and *D. miranda*, while in other cases the extreme links of a long chain of subspecies are

more different from each other than are most related species. Rensch (1929, 1938, 1939a) has pointed out that nearly every morphological character which has been used to separate species may also vary geographically within the species. Furthermore, morphological characters often vary independently from fertility and are therefore of no value in the all-important border-line cases. Morphological characters are, therefore, of no decisive value in a species definition, because *there is no difference between subspecific and specific characters*.

(b) *Genetic distinctness*: In the earlier years of the science of genetics, great stress was laid on the genetic distinctness of two species. Now we know, not only that all subspecies are genetically different, but also the populations within the subspecies (Dobzhansky for *D. pseudo-obscura*, Sumner and Dice for *Peromyscus*, Goldschmidt for *Lymantria dispar*, etc.). In fact every individual is a different biotype. We readily admit this in regard to man and domestic animals and plants, but it is equally true for all other animals. Genetic distinctness, being a *sine qua non* condition, is therefore of little value in a species definition.

(c) *Lack of hybridization*: This criterion is only of limited value. If no hybridization is possible between two neighboring populations, then there is little doubt that they are good species. The opposite condition is, however, not an absolute necessity. Many good species of animals are capable of producing hybrids in captivity, but never interbreed in nature.

A successful species definition should not lay too much stress on any one of the three above-listed criteria. Of all the definitions that have come to my attention within recent years, the one proposed by Sewall Wright (1940) seems to show the fewest flaws. According to him species are "groups within which all subdivisions interbreed sufficiently to form intergrading populations wherever they come in contact, but between which there is so little interbreeding that such populations are not found." This

definition again omits reference to those "subdivisions" which have the misfortune of not being able to come in contact with other subdivisions because they are spatially or ecologically isolated.

One might therefore propose the following emended definition: "*A species consists of a group of populations which replace each other geographically or ecologically and of which the neighboring ones intergrade or hybridize wherever they are in contact or which are potentially capable of doing so (with one or more of the populations) in those cases where contact is prevented by geographical or ecological barriers.*" Such a definition is applicable in practically all difficult cases of bird taxonomy except where the terminal links of a chain of races overlap (see below). It remains to be seen how useful it is when applied in other groups, although Remane (1927), who is not an ornithologist, arrived at a very similar formulation. As a taxonomist, I am, of course, interested in a practical definition, and a definition like Dobzhansky's is of little use in taxonomic work. Even the above given formulation is at best only an approach. In many cases of interrupted distribution it is necessary to leave it to the judgment and the systematic tact of the individual taxonomist, whether or not he considers two particular forms as "potentially capable" of interbreeding, in other words, whether he considers them species or subspecies. Furthermore, the just given definition applies to "biological or zoogeographical species." The taxonomist finds it sometimes useful for practical purposes to treat as full species what should be regarded as subspecies on the basis of the definition. Groups of such "semi-species" are called superspecies in the subsequent discussions (Mayr, 1931) (see also Rensch, 1934).

THE REALITY OF SPECIES

The discussion of various species definitions, having resulted in the conclusion that it was impossible to propose a completely satisfactory definition, must have

raised some doubt as to whether there is such a thing as species. As a matter of fact the opinion is very prevalent among non-taxonomists that "species" are just idealized abstractions, created by the taxonomist for his own convenience. This point of view may apply to the situation in little-worked groups of invertebrates, but it does not apply to the more thoroughly investigated systematic groups. The construction of a comprehensive, lucid species definition may present many difficulties, but in reality the species themselves are remarkably real natural phenomena (Dobzhansky, 1937).

A. INDIVIDUAL VARIATION AND SPECIES DELIMITATION

The taxonomist, in the course of his work, is generally confronted by two classes of difficulties when he tries to define the natural limits of his species. The first is caused by the evolutionary instability of species, and will be treated subsequently, while the second is caused by individual variation. The latter kind of difficulty is usually removed, as soon as sufficient material is available. It is quite amazing that in well-worked groups there is hardly ever any doubt what is a species and what not, even though there are no absolutely reliable species criteria in existence. In disputed cases the final decision usually shows that either (1) several stages or phases of a species are so different that they had been mistaken for different species or (2) just the opposite, that several species which occur in the same locality are so similar that they were considered stages of one species.

(1) *Phase confusion*: That stages or phases of one species are so different that they have been considered for a long time to belong to several species is a frequent occurrence in marine and fresh-water animals, as well as in insects. It is, however, also a common occurrence in birds. Linnaeus described the adult (= barred) plumage of the goshawk as *A. palumbarius*, the immature (= streaked) plumage of the same species as *A. gentilis*. I could quote dozens of similar cases where young and

adults or male and female were described as different species until closer study revealed the error. More difficult are the cases where strikingly different color phases occur in the same species, such as wild type and white birds, or wild type and melanistic, etc. Stresemann (1926) summarized (with colored plates) some of the more interesting cases. The number of such cases has been more than doubled in the last fifteen years and some highly unusual alternative plumages have been described (for example, tail-coloration in *Rhipidura brachyrhyncha*, Mayr and Rand, 1937). If such color phases are restricted to definite parts of the range of the species, particularly to the exclusion of the "wild" type, the difficulty is still greater. Two well-known "pairs" of species of North American birds seem to fall into this category (only 2 out of about 755!). The great white heron (*Ardea occidentalis*) is now considered specifically distinct from the great blue heron (*Ardea herodias wardi*), and the lesser snow goose (*Chen h. hyperboreus*) from the blue goose (*Chen caerulescens*), but I am confident that in both cases it will eventually be shown that the white partner of the pair is merely a color phase (mutant) which has become more or less restricted to a definite region.

(2) *Sibling species*: The opposite condition exists where pairs or larger groups of related species are so similar that they are generally considered as one species, or at least have in the past for a long time been mistaken for one another. Several such incompletely analyzed species groups are known from the genus *Drosophila*, for example, *Drosophila* "obscura" and "affinis." The species of the flycatcher genus *Empidonax* are the closest to this situation that we find among North American birds, although this case was completely settled more than a generation ago. From Europe two pairs of exceedingly similar species of birds are known, the two chickadees (*Parus atricapillus* and *palustris*) and the two creepers (*Certhia familiaris* and *brachydactyla*). Again

the differences in morphology, ecology, habits and distribution were largely cleared up more than forty years ago. So far as birds are concerned there are no more species difficulties of this sort in the better-explored parts of the globe. In the tropics and in the southern hemisphere there are a number of genera with extraordinarily similar species. Some of these have been recently monographed and the situation has been clarified; the most notable cases are the steamer ducks (*Tachyeres*) (Murphy, 1936), the grass warblers *Cisticola* (Lynes, 1930), the flycatcher *Cyornis* (several authors), the honey-eaters *Meliphaga analoga* and allies (Rand, 1936a) and the South American *Scytalophus* (Zimmer, 1939). There are, however, still a few genera left (for example, the cave swiftlet *Collocalia* with about sixteen species) in which only two or three specialists can even approximately identify the various species. Considering that there are about 8,500 species of birds in the entire world, the number of "difficult" species does not even amount to 1 per cent. The eventual elimination of all such cases may perhaps be considered the most tangible result of taxonomic work.

B. SPECIES IN THE "MAKING"

Another class of difficulties, however, will never be eliminated. We have in birds a high number of cases, where certain populations become detached from the main body of their "parent" species, by becoming isolated on an island, on a mountain or through some other geographical or ecological barrier, and develop characters during this period of isolation which deviate considerably from those of the parent species. Such "forms" (to use a neutral term) are geographical representatives of their next relatives and show the common origin still very clearly. Usually there is considerable taxonomic disagreement about these forms, the more radical authors calling them subspecies, the more conservative ones, good species. We fail miserably if we try to apply the test of species definition to such forms.

It is of interest to find out how common such cases are. I have made an analysis of all the North American birds listed in the A. O. U. Checklist (1931), a work which is rather conservative in its taxonomic point of view. I have omitted only introduced species and the purely marine order Tubinares. In 374 genera there are 755 species with a total of 1,367 species and subspecies. At least 94 of the listed 755 full species of North American birds will be considered by some authors to be merely subspecies of other species. In other words 12.5 per cent. of the species of North American birds have reached a very interesting taxonomic stage: They still show by their distribution and general similarity that they had been only recently geographical forms of some other species, but they have, in their isolation, developed morphological characters of such a degree of difference that the majority of authors now prefers to call them good species. Typical examples in the North American bird fauna are: Ipswich and Savannah sparrow (*Passerculus*), red-shafted and yellow-shafted flicker (*Colaptes*), Audubon's and myrtle warbler (*Dendroica*), the various species of the genera *Junco* and *Leucosticte*, etc. The majority of these forms are more or less isolated, either on the islands off the California coast or on the various mountain ranges of the Rocky Mountains or in the lowlands east and west of the Rocky Mountains. These "semi-species" comprise $12\frac{1}{2}$ per cent. of the total of species in the rather continental fauna of North America. For a typically insular region, namely the Lesser Sunda Islands, Rensch (1938: 275) thinks that not less than 47 species are intermediate among a total of 160 species. I have analyzed the birds of the Solomon Islands and find that if we employ a narrow species concept there are 174 species of land and fresh-water birds; if we, however, employ a wide species concept (= include within one species all geographical representatives) there are only 125 species. In other words, of 174 species there are 49 of intermediate status, that is 28.2 per cent.

I have used birds to illustrate this situation, because the highly refined condition of bird taxonomy permits the quotation of exact figures. The phenomenon as such is, of course, equally present in many other taxonomic groups. Goldschmidt (1937), for example, has called attention to the fact that many of Kinsey's "higher categories" of Cynipidae are nothing more than strongly evolved geographically representative forms. It is only "a matter of taste" whether we designate such isolated and distinct forms subspecies or species. No species definition has yet been found—and I am quite certain that none will ever be found—which will satisfactorily cover such cases.

But let us now examine under what conditions these border-line cases may have developed. Let us follow the entire course of the speciation process, as revealed by the sequence of the lower systematic categories. To do this we must pay particular attention to geographical variation.

GEOGRAPHICAL SPECIATION

Geographical variation is now generally considered to be one of the most important phenomena of evolution. The refinement of modern taxonomic research as well as the stimulus given by the development of genetics have had the result that population studies are very much in vogue, or as the taxonomist puts it "the comparison of series from various localities." This type of study usually leads to the conclusion that no two populations of a species are quite alike. Of the variation of a species of *Peromyscus*, Dice (1939) recently said:

The mice of no two localities are quite alike either in average body dimensions or in average . . . pelage color. Local variability of a similar kind occurs in all the species and races of *Peromyscus* which have been critically studied.

Such a statement would be valid in principle for nearly every species of mammal or bird and probably for all species of organisms. These were laboratory-bred animals reared under standard conditions and there is no

doubt that the variations among the local populations were of a genetic nature. These differences of the means, however, are merely of statistical value, and are not sufficient to enable us to identify individuals. Consequently, such populations will be united under one sub-specific name.

We sometimes unite even fairly distinct forms if the variants are distributed in a highly irregular manner. Any taxonomic character, such as pigmentation, patterns, proportions, etc., may show such irregular distribution, but it is most easily demonstrated if cases of simple size variation are selected. The accompanying map (Fig. 1)

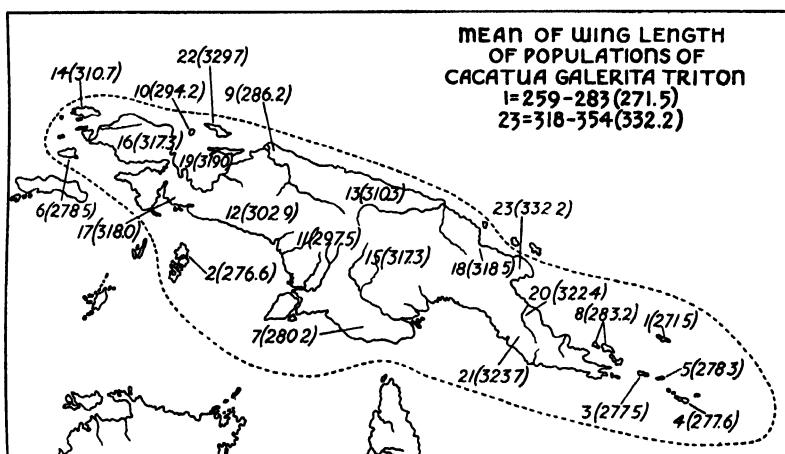


FIG. 1. Irregular variation within the range of one subspecies. The smallest and the largest of 23 populations are very different, but the gap is bridged by intermediate populations.

of 23 populations of the cockatoo *Cacatua galerita triton* (Mayr, 1937) and the table of 10 populations of the

IRREGULAR SIZE VARIATION OF THE HONEYEATER *Foulehaio carunculata* IN
CENTRAL POLYNESIA

	Males	Females
Ono Iau (E. Fiji) ¹	108-113 (110.0) ²	96, 97 (96.5)
Tonga Islands	104-114 (108.3)	92-101 (95.6)
Southern Lau Archipelago (E. Fiji)	103-110 (106.9)	89-98 (93.9)
Tutuila (Samoa)	102-109 (105.8)	91-97 (94.4)
Manua (Samoa)	99-107 (104.7)	92-98 (93.8)
Matuku (E. Fiji) ¹	104, 105 (104.5)	87-92 (89.3)
Upolu (Samoa) ¹	99-102 (100.8)	90, 93 (91.5)
Northern Lau Archipelago (E. Fiji)	99-103 (100.9)	84-91 (87.8)
Savali (Samoa)	93-102 (98.5)	85-92 (87.2)
Fotuna Island	93-98 (95.9)	82-87 (84.2)

¹ The measured series is too small to show the full range of size-variation.

² The figures indicate the length of the wing in millimeters.

honeyeater *Foulehaio c. carunculata* (Mayr, 1932) show that neighboring populations may be very dissimilar, while some very distant ones are practically indistinguishable. Sometimes in a chain of subspecies of one species, the alternate members are more similar to one another than the neighboring ones. The alternating races with cock or henfeathered females of the New Guinea wren-warbler *Malurus alboscapulatus* (Mayr and Rand, 1935) are a good illustration of this phenomenon.

Variation within continuous ranges: It does not concern us in this connection what the causes are of the genetic differences between populations. Chance losses of genes or alleles and fixation of new mutations partly in connection with selective forces of various sorts, are the factors that are usually quoted, although the belief in some kind of direct influence of the environment is still held by some taxonomists and will continue to be held in the future, until somebody finds convincing proof against it.

If we compare a series of neighboring populations or subspecies of one species, we often find that the differences between them are arranged in a graded series, for example, the easternmost form are palest, the westernmost darkest and all the in-between populations form a connecting series. J. Huxley has recently introduced the term *cline* for such a character gradient, but the phenomenon as such has been known and studied for more than a hundred years. The character gradient runs in most cases closely parallel to some climatic gradient and can be interpreted as being due to adaptive responses to selective factors in the environment. I shall not go into details since we have several admirable recent summaries (Rensch, 1936, 1939b; Huxley, 1939).

This interpretation can not, however, be applied when there is no evidence for an environmental gradient as, for example, in the case of some tropical clines known to me: *Rhagologus leucostigma*, *Paradisaea apoda* (see Fig. 2), *Gallicolumba* in Micronesia-E. Polynesia, etc. So far no

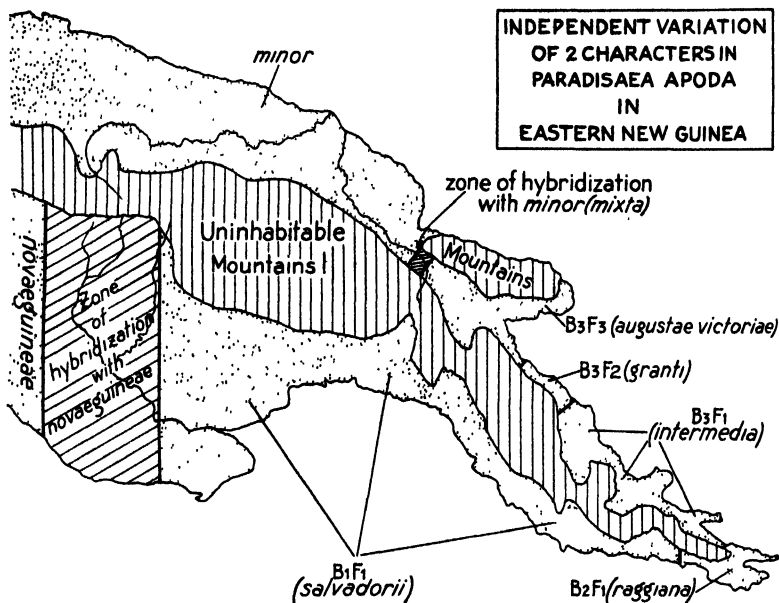


FIG. 2. A cline that is independent of environmental gradients. B. = Coloration of Back, B1, entire back brown, B2, upper back yellow, lower back brown, B3, entire back yellow. F. = Coloration of the plumes (flank feathers), F1, red, F2, orange-red, F3, orange.

successful interpretation is known to me, but instability of certain genes and gene loss in the border populations of an expanding species will have to be included in forming an interpretation.

We speak of irregular geographical variation wherever such character gradients (clines) are not discernible. Since most character gradients are correlated with climatic gradients, it is only natural that clines should be absent or little pronounced where species ranges are recent (selection has had no time yet to work) or where there are no environmental gradients, such as in wide parts of the humid tropics. Irregular variation, as is often found on tropical archipelagos, is very inconvenient to the taxonomist, as I have already pointed out in the cases of *Cacatua* and *Foulehaio*.

The phenomenon that an animal is evenly distributed throughout its range is an ideal that is practically never realized. Even the most wide-spread and common spe-

cies show gaps in their ranges and modern population studies have shown that the actual distribution picture resembles more that of clusters of little colonies than anything else. Even so there is usually so much interchanging of individuals between the neighboring and insufficiently isolated populations that the range is continuous for all practical purposes. In species with such continuous ranges we usually encounter the following phenomena (Goldschmidt, 1935; Kinsey, 1937a): (1) No clear-cut subspecies are formed. (2) Individual variation is high. (3) Each population is more or less intermediate between its neighbors. (4) There tends to be a gliding change of characters (clines), with an infrequent insertion of noticeable steps. (5) Some characters are curiously independent from the general trend within this range and show irregular variation. These points are of interest to the taxonomist, but they will also have to be considered by the geneticist. Species formation proceeds very slowly wherever such continuous ranges are involved and rarely goes very far.

The effect of discontinuous distribution: Up to now we have been dealing with fairly continuous ranges of populations, but *isolation* is required in addition to variation before significantly different forms can develop. This is not the place to discuss all the various isolating mechanisms that are found among birds; I shall restrict myself entirely to a discussion of the effects of *geographical isolation*. There is no case known in birds where geographical isolation was *not* the primary isolating mechanism. All the other isolating factors, such as ecological, psychological, cytological and genetic ones, can develop in birds only when a form is isolated and withdrawn from the interchange of individuals between neighboring populations. Such an isolation can occur either on true islands or other insular locations, such as on mountain ranges or in grassland islands within the tropical rain forest or in patches of forest within savannas, etc. (Birds are potentially good fliers and it might seem peculiar that

they should "stay put" on these distributional islands. I have quoted in a recent paper (Mayr, 1933) a number of cases of the effectiveness of natural barriers and the consequent isolation. A more detailed discussion is given by Stresemann (1927-1934, p. 635). A distribution map of a group of closely related species of *Zosterops* will illustrate this point better than any words could do (Fig. 3). Another excellent illustration of this phe-

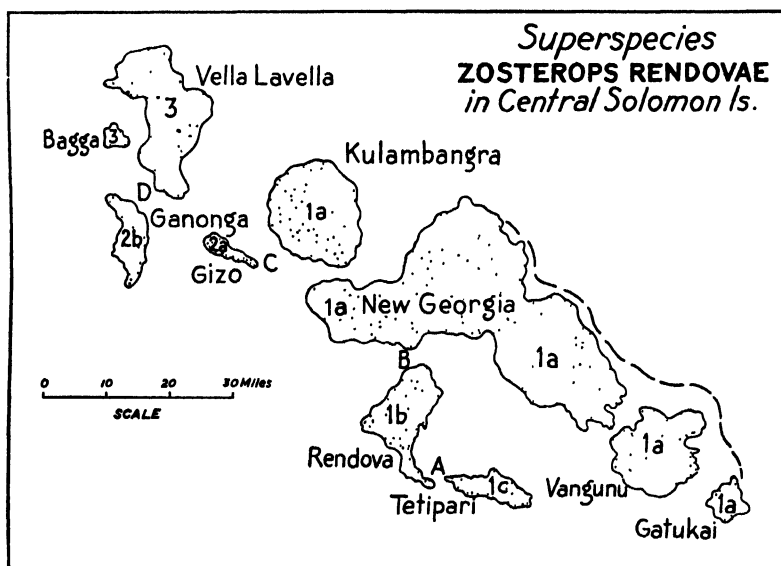


FIG. 3. Extreme localization of related species and subspecies in a tropical archipelago. 1 = *Zosterops rendovae* + subsp., 2 = *Z. z. luteirostris* + subsp., 3 = *Z. z. vella-lavella*. A = 1.7 km., B = 2 km., C = 6 km., and D = 5 km.

nomenon is the map of the distribution of the closely related species of the flycatcher *Pomarea* in the Marquesas Islands (Murphy, 1939).

The effect of such isolation is invariably speciation in all its stages. Some of the factors which determine the degree of differentiation are the following, four of them external and one internal.

External

- (1) Age of the island,¹ that is, the time interval since settlement by the particular species.
- (2) Size of the island, that is, size of the effective breeding population.

¹ Island in this connection means distributional, not geographic island.

- (3) Amount of competition, predation and other retarding selective factors.
 (4) Effectiveness of isolation, that is, amount of pollution by subsequent immigrants of the same species (swamping).

Internal

- (5) Inherent stability *vs.* mutability of the species.

Unfortunately, no studies are available, in which all these factors have been analyzed for a single island nor in a comparative manner for a group of islands. A forthcoming paper by D. Lack (1940) sheds some light on the relative importance of these factors among the finches of the Galápagos Islands. I myself (with de Schauensee, 1939) have shown in purely descriptive manner that speciation is a continuous process. Speciation on the two oceanic islands of Biak (near New Guinea) and Rennell (near Solomon Islands) is illustrated by the following table (including only the resident land and fresh-water birds).

STAGES OF SPECIATION ON TWO OCEANIC ISLANDS

	Biak Isl. All birds	Rennell Isl. All birds	Rennell Isl. Passeres only
No differentiation	20 = 29%	7 = 21%	1 = 8.5 %
Slight differentiation	11 = 16%	7 = 21%	0 = 0 %
Endemic subspecies	20 = 43%	15 = 44%	7 = 58%
Endemic species	8 = 13%	4 = 12%	3 = 25%
Endemic genera	0 = 0%	1 = 3%	1 = 8.5 %
Total species	68 = 100%	34 = 100%	12 = 100%
Distance from mainland	60 km.	145 km.	145 km.

Similar tabulations could be made for mountain birds. The warm lowlands are effective barriers for animals which are adapted to the particular climatic and ecological conditions only found in the mountains.

If we take three rather isolated mountain ranges in New Guinea, the Arfak, Cyclop and Saruwaged Mountains, all three comparatively well explored, and analyze the passerine birds we find the following figures:

DEGREE OF ENDEMISM ON THREE NEW GUINEA MOUNTAINS

Mountain species Passeres	Arfak	Cyclop	Saruwaged
Not endemic	36 = 40.4%	21 = 72.4%	59 = 68.6%
Endemic subspecies	46 = 51.8%	8 = 27.6%	23 = 26.7%
Endemic semi-species ¹ ...	5 = 5.6%	0 = 0 %	3 = 3.5%
Endemic full species	2 = 2.8%	0 = 0 %	1 = 1.2%
Total species	89 = 100 %	29 = 100 %	86 = 100 %

¹ Under semi-species, I understand, in this connection, a species which replaces geographically some other species, but is morphologically too different to be called a subspecies.

But even most of those species which are listed as "not endemic" are slightly different on each mountain range, only the differences are below the "taxonomic threshold."

BORDERLINE CASES

Well-isolated subspecies often diverge to such a degree that they are either on the borderline between subspecies and species or else that they are considered good species even by the progressive taxonomist although they still reveal their origin as subspecies. Four different classes of such borderline cases are in this connection of particular interest to us.

(a) *Ecological representation*: This phenomenon is common in insects and other lower animals, but rare in vertebrates. The ecological representation of two subspecies of *Peromyscus* is often quoted, but there is, so far as I know, nothing exactly comparable in birds. The closest approach is perhaps that of *Melospiza melodia* (song sparrow) of California where along San Francisco Bay one subspecies is restricted to the salt marsh and another one, which is very distinct, to the dry hillsides. In this case the ecologically representative forms are still subspecies. In another case the ecological representatives are now species and even though morphologically very similar, have different voices and other habits: The king rail (*R. elegans*) of the eastern United States inhabits fresh-water marshes and the clapper rail (*R. longirostris*), salt marshes. Many cases from the Palearctic Region have been recorded by Dementiev (1938).

Similar conditions seem to prevail in South Australia, where the wooded Mt. Lofty Range with its endemic races is like an island in the savanna with its races of the same species (*Sericornis frontalis*, *Acanthiza pusilla*). Actually, this case is not strictly ecological but also altitudinal representation. That birds of higher altitudes in the same mountain range belong to a different subspecies than the birds of the same species at a lower altitude is

not uncommon, particularly in certain parts of the tropics where birds are highly sedentary on account of ill-defined seasons. There is usually a gradual change from the lowland to the high-altitude population, but occasionally there are definite breaks, such as have been described from eastern New Guinea between *Philoprora guisei guisei* and *perstriata*, and between *Melidectes belfordi belfordi* and *brassi* (Rand, 1936b). Both *perstriata* and *belfordi* are well-defined races which are restricted to the mountain tops above 3,000 m, and whose range is now broken up into a number of islands, separated at a lower altitude by the continuous range of *guisei* and *brassi*. Similar cases have been described from the border of Tibet and Szetchuan, where the fauna of the Hsifan Mountain Forest and the Tibetan Plateau meet. *Phylloscopus fuscatus fuscatus* and *weigoldi* and *Ph. affinis* and *subaffinis* (Ticehurst, 1938: 89) are examples from this region. It is very probable that the ecological isolation was preceded in all these cases by geographical isolation. Theoretically in the same class are many of the highland races of the Roraima-Duida Plateaus of South America, which although often only separated by a cliff of 3,000 feet, are as different from their low altitude representatives as good species and are treated as such by the more conservative taxonomists (Chapman, 1931).

(b) *Extreme morphological development*: Populations of the same immediate common origin, but replacing each other geographically are, if different, generally called subspecies by the modern taxonomist. The old-fashioned criterion of intergradation by actual contact or (in cases of isolation) by individual variation is no longer demanded. But there are some cases where even the most inveterate "lumper" stops. They are the cases where certain subspecies, during periods of long and efficient isolation, have developed such a degree of difference that *they might be considered different genera if only morphological criteria were used. As a matter of fact, a great many isolated forms have originally been described as*

different genera and it is only recently that their true systematic position has become clear. I am illustrating it with the case of the barking pigeon (*Ducula pacifica*) of Polynesia, which has developed a form (*galeatus*) on the Marquesas Islands, which on account of its peculiarly developed bill was, until eight years ago, considered a good genus (*Serresius*) (Fig. 4). Other genera that are

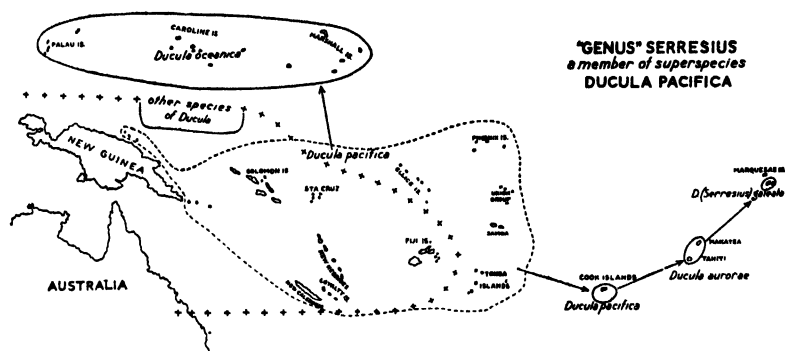


FIG. 4. Extreme differentiation of the most isolated form in the *Ducula pacifica* group.

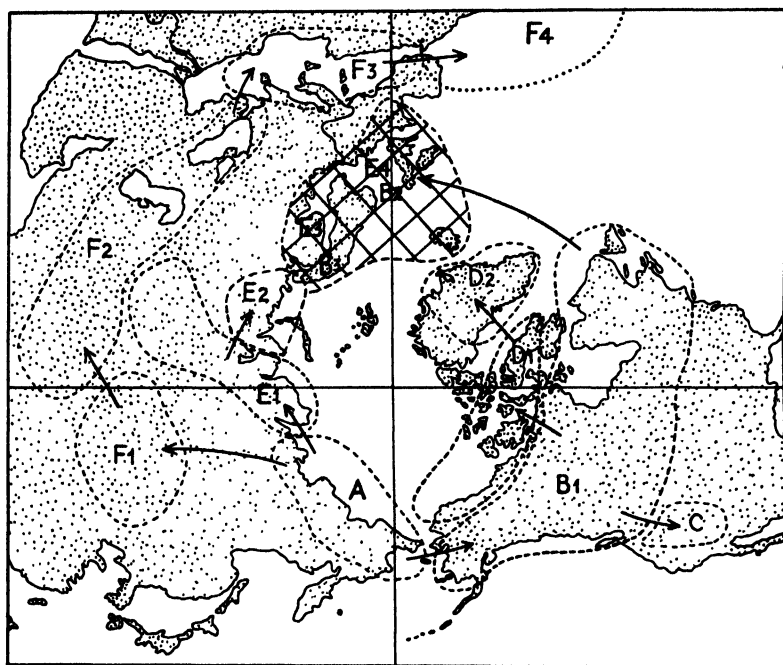
based on morphologically distinct geographical forms are: in pigeons—*Oedirhinus* (of *Philonopus iozonus*). *Chrysophaps* (of *Chrysoena luteovivens*); in kingfishers—*Todirhamphus* (of *Halcyon chloris*); in birds of paradise—*Astrarchia* (of *Astrapia nigra*), *Schlegelia* (of *Diphyllodes magnificus*), *Uranornis* (of *Paradisaea apoda*); in drongos—*Dicranostephes* (of *Dicrurus bracteatus*); in rails—*Porphyriornis* (of *Gallinula chloropus*); in Passeres—*Galactodes* (of *Erythropygia*), *Conopoderas* (of *Acrocephalus*), *Pinarolestes* (of *Clytorhynchus*), *Papuoorthonyx* (of *Orthonyx*), *Allocotops* (of *Melanocichla lugubris*), etc.

In most of these cases, and they are just a small selection from the numerous cases known to me, subspeciation, that is geographical variation, has actually led to the evolution of unquestionably new species. Some of Kinsey's species of Cynipidae apparently belong to the same class. The most strongly divergent forms, that is the most specialized or most recent ones, develop invari-

Mayr, 1933). Not a single case is known to me from recent islands, such as England or Ireland. On the other hand, the fauna of ancient archipelagos, like Hawaii and Galápagos, is composed of whole clusters of such species. In many cases they are still so similar to one another that they would probably be considered subspecies if they occurred on neighboring islands instead of on the same island.

The just quoted cases are the successful ones. Subsequent invasions originating from the parental stock are probably much more frequent than these few cases indicate, but the new settlers are quickly absorbed by the daughter population, because the latter had not yet developed into a new species. If entire subspecies meet on intermediate islands, complete hybrid populations result, such as I have described for *Megapodius* (Mayr, 1938) and in two cases for the genus *Pachycephala*.

(d) *Overlap*: The perfect demonstration of speciation occurs, of course, when a chain of intergrading subspecies forms a loop or an overlapping circle, in which the forms which co-exist in the same locality no longer interbreed. There is no better way than this to demonstrate the effectiveness of geographical variation. Unfortunately, there are not many complete cases. The best one in birds is probably that of *Parus major* (Rensch, 1933). Another good case is that of the herring gull group (*Larus argentatus*) (Stegmann, 1934). The races of this species are arranged in a circumpolar ring, but *Larus argentatus*, coming from America, invaded western Europe and lives now side by side with *Larus fuscus*, like a good species, although the two "species" are connected by a chain of intermediate forms in Siberia and North America (Fig. 6). Not quite so clear is the case of *Halcyon* in Micronesia (Fig. 7). Another interesting case, relating to two species of *Zosterops* in the Lesser Sunday Islands, was described by Stresemann (1939). The two honey buzzards of the Philippines (*Pernis celebensis steerii* and *Pternis ptil. philippensis*) are also con-



OVERLAP OF THE TERMINAL LINKS OF A CHAIN OF RACES OF *LARUS ARGENTATUS*

FIG. 6. Circumpolar projection of the ranges of forms of the *Larus argentatus*-group. A = vegae, B1 = smithsonianus, B2 = argentatus, B3 = omisus, C = californicus, D1 = thayeri, D2 = leucopterus, E1 = heuglini, E2 = antelius, E3 = fuscus, E4 = graellsii, F1 = mongolicus, F2 = cachinnans, F3 = michahellis, F4 = atlantis. *L. fuscus* (+ *graellsii*) lives now like a good species besides *L. argentatus* (+ *omisus*).

nected in Malaysia by a ring-like chain of intermediate forms.

THE STAGES OF GEOGRAPHICAL SPECIATION

The point which I have tried to illustrate in the preceding discussion is that there is every condition existing between an almost uniform species and one in which isolated populations have diverged to such a degree that they can just as well be considered as separate good species. The tabulations of the level of speciation reached by various species on isolated oceanic islands (p. 267) and in mountain ranges (p. 267) show clearly that geographical speciation is by necessity linked up with and influenced by geographical factors. In consequence, the

actual process that leads to the development of new species, the time required for it, etc., is somewhat different not only in every species, but also in every geographical district. A worker who confines his studies to one particular region may not and will not get a complete picture

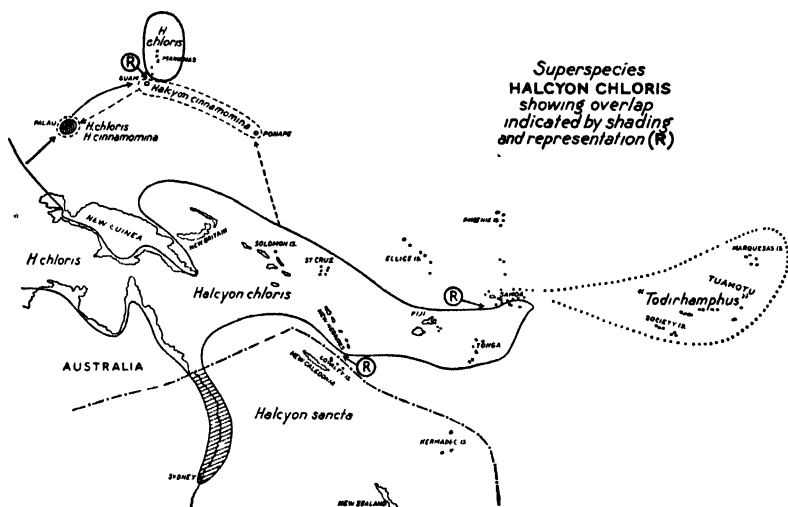


FIG. 7. The closely related kingfishers of the superspecies *Halcyon chloris* still replace each other geographically (R), except on two places: Palau Island and the coast of eastern Australia. In both locations two forms live side by side like good species.

of these phenomena. I have been thinking how I could best demonstrate this point and have arrived at this solution.

It is possible to generalize and simplify the diversified phenomena of evolution and to classify all species of birds according to the degree of speciation which they have reached. Such a classification is based on a simplified outline of the process of evolution, for example, as follows:

- Stage 1: A uniform species with a small range
followed by
Process 1: Expansion of range
resulting in
- Stage 2: A uniform species with a large range
followed by
Process 2: Differentiation into subspecies
resulting in

Stage 3: A geographically variable species with a more or less continuous array of similar subspecies (3a all subspecies are slight, 3b some are pronounced)

followed by

- Process 3a and 3b: Development of geographical barriers between some of the populations; also: Development of isolating mechanisms in the isolated and differentiating subspecies

resulting in

Stage 4: A geographically variable species with many subspecies completely isolated, particularly near the borders of the range, and some of them morphologically as different as good species

followed by

- Process 4: Expansion of range of such isolated populations into the territory of hitherto strictly representative forms resulting in

Stage 5: Overlapping ranges of closely related species either of which might be in any of the stages 1-4.

The taxonomist will be principally interested in stages 1-5, the ecologist and zoogeographer in processes 1, 3a and 4 and the geneticist in processes 2 and 3b. Species in nature are, of course, not always clearly referable to one or the other class, as I have defined them above. Not only are some of them between two stages but also very often one half of the range of a species is in one stage, the other half in another stage. There are also those cases of ring-like chains of forms which are difficult to fit into the above given classification.

It is possible, in spite of all these difficulties and objections, to take the entire number of species of a systematic group (let us say birds or butterflies) from one particular region and divide them up according to the stage of speciation to which they belong. (Stage 5 will have to be treated separately, because overlapping species will be considered as full species and be listed in classes 1-4.) The resulting figures of such an analysis shed much light on the degree of speciation in the respective regions. To demonstrate the value of this method, which is only applicable to thoroughly well-known groups, I am tabulating the passerine birds of three geographical regions, including the extra-territorial range of each species. For

practical reasons I have subdivided species with weak (3a) and with pronounced (3b) subspeciation.

DEGREES OF SPECIATION IN DIFFERENT GEOGRAPHICAL REGIONS

Stage	Manchuria ¹ (continuous ranges)	New Guinea region (partly continuous ranges)	Solomon Islands (discontinuous ranges)
	No. of species	No. of species	No. of species
1	2 = 1.9%	34 = 11.7%	9 = 18%
2	15 = 14.0%	21 = 7.2%	1 = 2%
3a	59 = 55.1%	118 = 40.7%	11 = 22%
3b	30 = 28.0%	84 = 29.0%	12 = 24%
4	1 = 1.0% ²	33 = 11.1%	17 = 34%
Total	107 = 100 %	290 = 100 %	50 = 100%

¹ Including all relatives within the Holarctic Region.

² Actually this is a case of discontinuous distribution, because it relates to a representative species on the Bonin Islands.

An analysis of this tabulation shows that stages 4 and 1 which indicate the final stages of evolution, are practically non-existent where geography and geology favor continuous ranges. On the other hand, stages 2 and 3a, indicating the early stages of evolution, reach a definite high in such continental areas. In contradistinction we find that where geographical factors break up the species ranges to a high degree, as, for example, on an old tropical archipelago such as the Solomon Islands, a great number of the species are in the final stages of evolution (4 and 1) and comparatively few in the early stages (2 and 3a). A student of speciation must study both regions with continuous and discontinuous ranges before he can generalize on the dynamics of the speciation process. To base all conclusions on the Palearctic or the Nearctic regions leads inevitably into error, because these two regions show special conditions: There are few effective geographical barriers and many of the present ranges were comparatively recently occupied, that is after the retreat of the ice. All the workers who believe that geographical variation does not lead to species formation, base this opinion on research done in the Holarctic Region. However, I believe that I have quoted sufficient examples to show that geographical variation does lead to formation of new species, provided that it is coupled with isolation.

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These symposia will be concluded with contributions by Dr. Lee R. Dice, Professor Warren P. Spencer and Professor Th. Dobzhansky in the July-August issue.

SHORTER ARTICLES AND DISCUSSION

A STATISTICAL STUDY OF TWO VARIABLES IN THE SEQUOIAS—POLLEN GRAIN SIZE AND COTYLEDON NUMBER

MORPHOLOGICAL characters commonly utilized by the taxonomist have often of necessity dealt with maxima and minima in size range or numerical range. These are very useful in indicating the spread of variations, and when they do not overlap similar characters in other species or genera, the general results are adequate. If there is a slight overlap between two related forms a more statistical treatment of the data is indicated.

It is the purpose here to show that the two variables found in pollen grain size and in cotyledon numbers, in which the extremes overlap slightly, are of the greatest significance when analyzed by statistical methods. By such treatment the mean values of random samples within sufficiently wide range are consistent, and extreme differences, when present within related genera, become at once apparent. The analyses need involve only the more simple biometric methods which are in common use (Davenport and Ekas, 1936). Means, standard deviations, standard errors, etc., are easily obtained after the routine counts are made. If the amount of material is limited there is naturally greater opportunity for discrepancies in results, but standard errors prove valuable in such cases. Just what constitutes a random sample is answered at least in part by the results of this type of treatment. The justification for letting the constants of such a sample represent the whole rests, of course, on the laws of probability.

On the basis of external morphology, the pollen of the Giant-sequoia or Big Tree *Sequoiadendron giganteum* (Lindl.) Buchholz (1939) and the Redwood, *Sequoia sempervirens* (Lamb.) Endl., is similar. Wodehouse (1935) reports the pollen to be indistinguishable in the two genera. The germinal papillae appear alike, and both have pollen with typically thin exines and thick intines. The intine swells considerably, even to the extent of bursting and thus shedding the exine if placed in water, and also in glycerin jelly if not carefully treated. Wodehouse's (1933) methyl-green-glycerine-jelly method was used in order to obtain fully expanded grains. The methyl-green stains the exine but not the intine, thus providing good contrast.

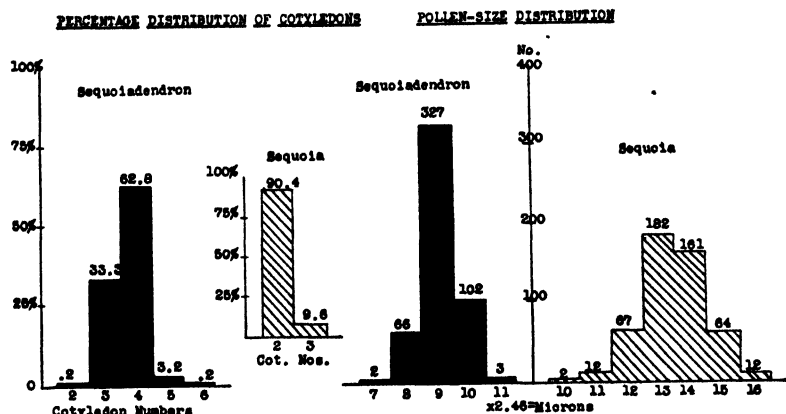
Examination of the pollen of these two genera, disclosed marked differences in size. From measurements of 500 expanded grains of each genus there appears a slight overlapping of the maximum size of pollen of *Sequoiadendron* with the minimum size of pollen of *Sequoia*. However, the mean size of the former is $22.47 \pm .05$ microns, whereas that of the latter is $32.85 \pm .11$ microns. The difference in the two means is 85 times the standard error of the difference and is of significance. Standard deviations in these counts likewise show differences, *Sequoiadendron* being $1.47 \pm .05$ microns and *Sequoia* $2.60 \pm .08$ microns. Here again the difference is significant, since a difference of more than twice the standard error is obtained, in this instance 12 times the standard error of the difference. These differences are not due to the random sampling method employed, but may be regarded as size distinctions between these two genera, possibly contingent upon the fact that *Sequoia* is a polyploid (J. T. Buchholz, 1939) whose pollen diameter may be expected to be larger than that of *Sequoiadendron*.

Using the mean diameters of both genera the volume of the *Sequoia* pollen grain is calculated to be over four times that of *Sequoiadendron*. This, however, is an approximation, since the grains are not perfect spheres, but the data tend to show a relationship similar to that found in the *Daturas* (Blakeslee, Belling and Farnham, 1923).

The two graphs showing pollen distribution are based on total counts of 500 grains in each genus. From these graphs one obtains at a glance the spread or range in sizes, the overlapping at the extremes, and can approximate the average size of the grains. The spaces on the abscissa if multiplied by 2.5 will give the diameter of the pollen grains in each category. At about 25 microns there is an overlapping in size of grains of the two genera. Most of the pollen counted of the Big Tree is found to be 22 microns in diameter. This agrees rather closely with the mean obtained ($22.47 \pm .05$). In the Redwood the greatest number of grains measure 32 microns. The mean in this instance is $32.85 \pm .11$ microns. This figure is slightly larger, but is due no doubt to the large number of grains falling within the 34.5 micron group to the right of the 32 micron group.

Number of cotyledons when reported in the literature may sometimes list minimum to maximum ranges but mean values are usually neglected. In Gordon's "Pinetum" (Gordon, 1880) the range for the Big Tree is given as 3-6 (mostly 4's) and the Red-

wood is given as 2's mostly, sometimes 3 cotyledons; Hickel (1911) in his extensive report on seeds and seedlings of conifers lists the Big Tree as having ordinarily 4 (rarely 3 or 5) cotyledons, and the Redwood is given as 2, exceptionally 3; Pilger (1926) indicates (erroneously) a range of 4-6 for both of the sequoias; but from none of these has the reader any idea how many or how few specimens were actually examined.



Graphs showing distribution of cotyledon numbers and pollen grains in the two sequoias.

A statistical study similar to that for pollen was made of the number of cotyledons of embryos of the two genera. Of 366 embryos counted *Sequoiadendron* shows an extreme range of 2-6 cotyledons with a mean value of $3.69 \pm .03$ cotyledons, whereas *Sequoia* on the basis of 248 embryos has a mean of $2.09 \pm .02$. Although the cotyledon numbers of the two genera overlap at the extremes, the difference shown is 50 times the standard error of the difference and is significant. Standard deviations also show differences, being $.52 \pm .02$ in *Sequoiadendron* and decidedly less in *Sequoia* ($.29 \pm .01$). The standard error in this case is 10 times the standard error of the difference.

Two *Sequoia sempervirens* embryos showed partial fusion of these organs, simulating a condition of cotyledonary tube formation. Another abnormality noted in this genus was found in one embryo which had a fourth cotyledon as a small protuberance arising from the region of the plumule but slightly displaced into the cotyledonary node and which was much smaller than the three average-sized cotyledons.

The graphs show the distribution of cotyledon numbers on a percentage basis, since the total number of embryos counted in each case is not the same. The Big Tree (*Sequoiadendron*) shows a range from 2-6 and not 3-5, 3-6 or 4-6, as previously reported by various writers. Over half of the embryos observed (62.8 per cent.) showed 4 cotyledons. The mean is $3.69 \pm .03$ when counts are treated statistically. By far the majority of the Redwood (*Sequoia*) embryos had but 2 cotyledons (90.4 per cent.) and the mean obtained is $2.09 \pm .02$, as indicated before. The one abnormal embryo with 4 cotyledons is not included in the data nor shown on the graph, since the small fourth cotyledon arose from a higher point. It was a plumular leaf which made its appearance early.

Thus there is afforded by this type of treatment a more exact statement of measurable differences in terms of constants; it places these differences on a comparable basis; it discloses significant differences when present and is particularly valuable when extremes overlap, such as they do in these two variables of cotyledon number and pollen grain size.

Comparison of dry weights of embryo and endosperm in *Sequoia* in a dozen cases show the embryo to be 36.3 per cent. of the total dry weight; in *Sequoiadendron* the embryo constitutes 40.7 per cent. of the total dry weight. The difference here is small, is based on a very small sample, and is probably not significant. It may be of interest in connection with the work of Clare and Johnstone (1931) on certain pines.

One cone of *Sequoia sempervirens* obtained from cultivation in the Santa Clara Valley yielded a total of 16 embryos, all with 2 cotyledons. This particular yield is much above that obtained from any other locality.

Certain commercial seeds of *Sequoia sempervirens* (collected by Eustice Rush) consisted of only 3 per cent. good seeds. At the rate of 60-80 ovules per cone these would represent a yield of only 1.8-2.5 good seeds per cone. Whether we consider the cone yield in seeds as 16 good seeds per cone (20-26 per cent.) or only 2-3 seeds per cone estimated from the commercial seeds, this rate of seed abortion is extreme in this species and is probably due to the tetraploid condition found in the Redwood.

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INDUCTION BY FAST NEUTRONS OF MUTATIONS IN *ANTIRRHINUM*: II. GENETICS¹

PREVIOUS papers² have reported the effects of fast neutrons on the dry seeds of *Antirrhinum*, *Oenothera*, *Myosotis*, *Ripsalis*, *Echinocereus* and *Neomammillaria*.

In the first paper it was shown that perfectly dry seeds of members of the above genera when indirectly bombarded with fast neutrons resulted, in a number of cases, in decreased germination as well as in marked changes in general morphology upon subsequent planting.

The second paper described how plants grown from the *Antirrhinum* seeds gave rise to flowers which in two cultures were markedly unlike the parent plants from which the seeds were originally obtained. A third culture showed no changes whatsoever with respect to color.

It is the purpose of this paper to record the results of genetic work done on plants grown from seed from the above-described cultures.

Representative plants from each color group in the two cultures showing anomalies were selected for the present work.

¹ Research conducted at the University of Michigan in the departments of botany and physics.

² R. M. Chatters, *Science*, 87: 262-263, 1938; *Science*, 88: 241-242, 1938; *Chronica Botanica*, 4: 391, 1938.

These plants were self-pollinated and the flowers covered with glassine bags to prevent contamination by insect- or wind-borne pollen. The majority of these selected plants failed to set seed.

Plants in Culture No. 2, the progeny of which are being discussed in this paper, were grown from seed which came not only from the same plant but from the same seed capsule. This original plant bore cerise (rose pink) blossoms only.

Two plants which did set seed that later proved to be viable were No. 2/45 and No. 2/69. The former bore burnt orange (called orange-bronze by the writer) and the latter cerise (rose pink) colored blossoms.

Upon planting seeds from these two specimens it was found that many of the seedlings showed the tendency to be dwarfed, weak, and to have mottling on the leaves, as was the case in the parent culture.

Samples of leaves from these new plants were sent to Dr. O. L. Inman, of the C. F. Kettering Foundation at Antioch College, to be tested for any chemical change in the chlorophyll which might possibly have resulted from the bombardment. As in the case of leaves from the parent plants, the results were negative.

Of 100 plants grown from seed obtained from No. 2/45, there were 48 plants bearing burnt orange flowers, and 52 were cerise.

In the case of No. 2/69 there were 74 plants which grew to maturity and flowered. Of this number 21 were burnt orange and 53 cerise.

All the data with respect to color changes due to the bombardment by fast neutrons are summarized in Table I. It is evident

TABLE I

Original <i>Antirrhinum</i> plant	Flower color after irradiating seeds	Color of progeny after self pollination
Culture No. 1 Yellow (lemon yellow)	No change	Not continued
Culture No. 2 Cerise (rose pink)	cerise 48.2 per cent.	} burnt orange . 48 per cent. } rose pink 52 " " } no progeny } " " } " " } cerise 72 " " } burnt orange . 28 " "
	pink "5T" 5.5 " "	
	pink "1T" 0.8 " "	
	magenta 1.6 " "	
	burnt orange . 34.7 " "	
	bittersweet ... 9.2 " "	
Culture No. 3 Burnt orange	burnt orange . 54.1 " "	not continued
	bittersweet ... 29.1 " "	no progeny
	yellow "Li" .. 4.1 " "	" "
	spinel rose ... 12.7 " "	" "

from these data that (1) it is not only possible to produce (or

hasten, as one will) mutations by means of fast neutrons, but that (2) these changes may result from the irradiation of *perfectly dry seeds*. Also (3) it is further evident that these mutants after self-pollination will, when viable, produce like progeny in a sufficiently large number of cases as to appear to be significant.

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THE ORIGIN OF AMERICAN TETRAPLOID GOSSYPIMUM SPECIES¹

DENHAM (1924) found the species of *Gossypium* to have either 13 or 26 pairs of chromosomes. The 26-chromosome group is composed of American cultivated cottons and several closely related wild species, which are indigenous to Mexico, South America, Galápagos Islands, and Hawaiian Islands. The 13-chromosome species, which includes cultivated Asiatic cottons, and wild species from Asia, Africa, Australia, Central America, and the Galápagos Islands (Harland, 1939) can be separated into five distantly related types.

Skovsted (1934, 1937) advanced a hypothesis, on evidence derived from chromosome pairing in hybrids and chromosome size, that 26-chromosome *Gossypium* species were allotetraploids with one of the original species similar to Asiatic and the other similar to American 13-chromosome species. Gates (1938) and Webber (1939) were unwilling to accept fully Skovsted's hypothesis, and they considered other possible origins of the tetraploid type.

Obviously, more conclusive evidence of Skovsted's hypothesis could be secured by producing tetraploids that combine American 13-chromosome with Asiatic 13-chromosome species. In the present work tetraploids of this type were produced by doubling the chromosome number in hybrids of *G. therberi* Tod. \times *G. arboreum* var. *neglectum* Hutchinson and Ghose.

The synthesized tetraploid is highly female fertile, but usually the pollen is non-viable. One flower had an abundance of functional pollen which caused seed to develop on the tetraploid and also in crosses with the natural tetraploid form. Hybrids are easy to produce between the synthesized tetraploid and natural tetraploids by using the latter as the male parent.

¹ This work was done while the author was agent, Bureau of Plant Industry, United States Department of Agriculture, Raleigh, N. C.

During meiosis in hybrids between the original species less than half of the chromosomes pair. The bivalents usually gave bridges at anaphase, which is evidence that structural differences existed between all the chromosomes of the two species. After the chromosome number is doubled in the hybrid most of the chromosomes pair, but sometimes multivalents and one or more univalents are present.

The meiotic chromosome behavior in hybrids between the synthesized tetraploid and natural tetraploids is what would be expected according to Skovsted's hypothesis. In meiosis of the hybrid there are usually 24-25 pairs of chromosomes, which leaves 2-4 univalents. In some cells all the chromosomes are paired; there are, however, usually one to three or more multivalent associations. The hybrids will set seeds when self-pollinated, and seeds are produced in reciprocal crosses with the natural tetraploid type. These facts leave no doubt that the American 26-chromosome cottons are allotetraploids, with one parent species similar to existing American 13-chromosome species and the other similar to Asiatic 13-chromosome species. The synthesized tetraploid can be classified as a separate 26-chromosome species, but it is similar in origin and closely related to the natural 26-chromosome species.

An unusual fact about the American tetraploid *Gossypium* species is that one of the parent types is indigenous to the New World, while the other is indigenous to the Old. There can be no doubt that the tetraploid type is pre-Columbian, and Harland (1939) states that the type probably arose in late Cretaceous or early Tertiary.

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TEXAS AGRICULTURAL EXPERIMENT STATION

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THE SYMBOLIZING OF GENES AND OF CHROMOSOME ABERRATIONS

At the International Congress of Genetics held at Ithaca (N. Y.) in 1932 it was resolved that the genetical societies of all countries should cooperate to prepare recommendations regarding the problem of standardizing genetical symbolism in order to discuss them at the next International Genetical Congress. The International Committee of Genetical Congresses appointed Professor Dr. Tine Tammes (Groningen University) to take charge of the work to be done on behalf of this resolution; in cooperation with Dr. H. de Haan, Miss Tammes prepared a preliminary report on symbolism. Afterwards Miss Tammes delegated the International Union of Biological Sciences to continue her task; in cooperation with the International Institute of Intellectual Cooperation at Paris, the Union convoked a meeting of delegates from various countries, which meeting was held in London in the hospitable home of the Linnean Society on August 14 and 15, 1939. This meeting, under the presidency of Professor Dr. M. J. Sirks (Groningen) was attended by the following delegates: Dr. A. Establier and Miss N. Nicolsky (from the I. I. I. C. at Paris), Professor Dr. Ö. Winge (Denmark), Dr. B. Ephrussi (France), Professor Dr. H. Nachtsheim (Germany), Professor Dr. R. R. Gates, Professor Dr. J. B. S. Haldane and Dr. A. E. Watkins (Great Britain), Professor Dr. K. v. Körösy (Hungary), Dr. K. Ramiah and Dr. S. N. Venkatraman (India), Professor Dr. M. J. Sirks and Dr. S. J. Wellensiek (Netherlands), Professor Dr. O. L. Mohr (Norway), Professor Dr. M. Skalinska (Miss Skalinska, Poland), Dr. O. Tedin (Sweden), Professor Dr. F. Baltzer, Professor Dr. E. Hadorn and Professor Dr. A. Ernst (Switzerland) and Professor Dr. E. W. Lindstrom (U. S. A.); the delegates from Belgium, Italy, Finland and Japan being prevented from attending.

The following rules for symbolizing genes and chromosome aberrations have been drawn up:

Choice of a Standard Type. If it is desired to establish a standard type, this should be the most common form as a wild type or, if such can not be determined, the first studied most dominant form, wild or cultivated.

Symbols for Genes of Standard Type. Generally +; for definite genes preferably the gene symbol with + as a superscript.

Symbols for Other Genes. The smallest possible number of

the initial letters of the name for the character for which Latin is recommended when possible.

Indicating Dominant and Recessive. Dominant, capital initial letter; recessive, small letter.

Multiple Alleles. The symbol of the first discovered allele, in small letters if recessive to standard type, with a capital initial letter if dominant to it. The standard type is designated by the same symbol with + as a superscript; the others by the same symbol with a special superscript in capitals for dominant, in small letters if recessive as compared to the first allele.

Polymeric (Multiple) Genes. Genes which can not be distinguished by the effects and for which the loci are not known are symbolized by the same symbol with different Arabic figures as subscript A_1 , A_2 , A_3 or by the same symbol with the Roman figure of the chromosome as subscript A_I , A_{II} , A_{III} .

Lethal Genes are indicated by the Greek letter lambda (λ) which should be reserved for them, eventually as a subscript to another symbol or separate combined with an Arabian figure with a Roman figure for the number of the chromosome, both as a subscript (λ_1 , λ_2 , λ_3 IV, and so on).

Genes in Polyploids. When the chromosomes form polyvalents or if they pair at random, or if the segregation follows autopolyploid ratios, then the genes are written as many times as they are present, AAA_a , AA_{aa} , A_{aaa} , and so on.

Groups of Linked Genes. The genes written in order from left to right in the chromosome and the symbols spaced II A b c D; between those of different chromosomes a semicolon.

Genes in Related Species. The same symbol but with a subscript of the abbreviation of the specific name.

Formulae. Generally AABbCc, but if parental gametic genotypes are known ABc/AbC and if a linkage group is concerned and the loci of the different genes are known $\frac{a \ b \ c}{d \ e}$, the female gametic genotypes being mentioned first.

Reciprocal Crosses. In case plasmatic inheritance is involved an abbreviation of the name of the mother in parenthesis before the genotypic formula.

Priority shall be valid if no essential objection to the symbol can be made.

Lettertype. Italic for symbols of genes; Roman letters for chromosome aberrations and rearrangements.

CORRESPONDENT

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THE RELATION OF GENETICS TO GEOGRAPHICAL DISTRIBUTION AND SPECIATION; SPECIATION. II.*

SPECIATION IN PEROMYSCUS¹

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THE summary of the investigations on speciation in the mice of the genus *Peromyscus* here presented covers studies conducted by myself and my associates over a period of sixteen years. Investigations of *Peromyscus* were begun much earlier by Dr. Francis B. Sumner, and I have had the advantage not only of the experience of Sumner, but also of the use of his stocks, which he kindly turned over to me at the conclusion of his work with this genus. Much of the field work has been supported in the past by the Carnegie Institution of Washington, through the kindly interest of Dr. John C. Merriam, who until recently was president of the institution. The studies of *Peromyscus* at the University of Michigan were supported at first through the Museum of Zoology and later through the Laboratory of Vertebrate Genetics. The work at the university has been made possible through

* These three papers conclude the combined symposia given at meetings of the American Association for the Advancement of Science, the first on "The Relation of Genetics to Geographical Distribution and Speciation" at Milwaukee, Wisconsin, before Section F—Zoological Sciences, on June 20, 1939, and the second on "Speciation" at Columbus, Ohio, before a joint session of the American Society of Zoologists and the Genetics Society of America, on December 28, 1939, the first part of which has been published in the May-June, 1940, issue of *THE AMERICAN NATURALIST*.

¹ Read at a joint symposium on "Speciation" of the American Society of Zoologists and the Genetics Society of America, American Association for the Advancement of Science, Columbus, Ohio, December 28, 1939.

the interest and encouragement of President Alexander G. Ruthven.

Evolution within the genus *Peromyscus* has resulted in the differentiation of forms of varying degrees of distinctness from one another. These various forms may roughly be classified, according to the usage of mammalogists, as local races, subspecies or geographic races, species, species groups, and subgenera.

By local race I mean any local population whose characters differ to a recognizable degree from those of the other local races of the same subspecies. The local race as here defined is, I believe, the same thing as the microgeographic race of Dobzhansky (1937: 146-148). In *Peromyscus*, and probably in other animals, each more or less isolated intrabreeding local population (gamodeme of Gilmour and Gregor, 1939: 333) actually constitutes a local race, for the studies of Summer (1920: 370-371; 1932: 56-64) and myself have shown that no two local populations of *Peromyscus* ever have exactly the same characters.

The local races of *Peromyscus* differ from one another both in the dimensions of the body and skeleton and in pelage color. The differences in certain measurements between the populations of two nearby woodlots may cover nearly the extremes of variation of the particular subspecies concerned (Dice, 1937a: 17-20).

The complexes of characters which distinguish some local races are undoubtedly the result of chance, the combined effect of mutation rate and of the random elimination of genes (Dobzhansky, 1937: 118-148). The characters of other local races are correlated in part with certain features of the environment. In particular, the local races of *Peromyscus* tend to be relatively pale in coat color on pale-colored soils and dark in color on dark-colored soils (Dice, 1938: 16-18; 1939a: 19-23; 1939b: 13-16). The environment, therefore, through natural selection and possibly in other ways, may play a part in the formation of some local races.

The production of a local race or subspecies of any

kind of animal theoretically requires, as a necessary condition, some degree of isolation from the remainder of the population of the species. This isolation may sometimes, however, be only that produced by distance. The young of most small mammals do not travel very far, on the average, from their place of birth before taking up permanent residences. Accordingly, only a small amount of dispersal takes place in each generation, with the result that there is local inbreeding. That distance alone is an important barrier is demonstrated by the considerable number of subspecies of mammals which are separated from one another only by poorly defined areas of intergradation (Dice and Blossom, 1937: 116-117).

A more important barrier to free interbreeding within a species population than distance alone is the partial isolation caused by the unequal distribution of suitable habitats in nature. The most favorable habitats for a species often occur in patches which are considerable distances apart. Species, therefore, seldom are distributed uniformly over their geographic ranges, but tend instead to occur in more or less isolated colonies. Although there may be frequent interchange of individuals between nearby colonies, a certain amount of inbreeding must occur in each colony. This inbreeding, possibly aided by natural selection and mutation, theoretically will in time lead to the production of distinctive characters in each local population.

The greatest production of local races occurs where barriers are most effective in preventing free interbreeding between the several parts of the whole population of a species. On the deserts of southwestern North America, for example, the local colonies of the cactus-mouse (*Peromyscus eremicus*) on the desert mountains often are separated from one another by many miles of inhospitable desert plain. On these desert mountains many local races have been produced, the pelage color of each of which tends to match the soil color of its local habitat (Dice, 1939a: 19-23).

All the local races of each subspecies of *Peromyscus*

are, so far as we have been able to test them in the laboratory, fully interfertile. There seem to be no barriers to intercrossing between the several local races of a subspecies other than such physical or ecological barriers as may prevent free dispersal.

The subspecies, or geographic race, according to the usage of the term by vertebrate zoologists, is a larger unit than the local race. It is a division of a species made up of individuals which have certain characters in common and which occupy, usually to the exclusion of the members of other related subspecies, a definite section of the geographic range of the species. That the characters of certain subspecies of *Peromyscus* are inherited has been demonstrated by Sumner (1924). The subspecies is, however, not necessarily uniform in its hereditary constitution throughout its whole range and some subspecies are probably polyphyletic in origin (Dice, 1940). It is my opinion that the subspecies is best considered to represent an ecologic response, largely of an hereditary nature, expressed by those members of a species which live in a restricted but not always continuous geographic area having a certain type of environment.

The geographic races (subspecies) of *Peromyscus* have all, so far as they have been tested, proved to be completely interfertile in the laboratory with other geographic races of the same species. In nature, however, two subspecies of the same species may sometimes live in the same district, but fail to interbreed (Dice, 1931). A conspicuous example occurs in Glacier National Park, Montana, where a forest-inhabiting subspecies, *Peromyscus maniculatus artemisiae*, meets a grassland race, *P. m. osgoodi*, with no evidence of interbreeding (Adolph Murie, 1933). The failure of these two subspecies to interbreed in nature is due in part to a difference in their habitats (ecological isolation of Dobzhansky, 1937: 233). The complete separation of the two forms, however, evidently is dependent upon a difference in their mating reactions, for at some places near the margins of their habitats the two races live together without interbreed-

ing. This type of isolation has been called sexual isolation (Gulick, 1905: 84).

The morphological, ecological and psychological differences which separate the subspecies *artemisiae* and *osgoodi* must be assumed to have arisen at a time when the two were not in contact. Subsequently, the ranges of the two forms must have changed so that now the two subspecies meet, without interbreeding, in the Glacier Park region. The two subspecies still are connected together indirectly through chains of intergrading subspecies (Osgood, 1909, pl. 1, where *nebrascensis* = *osgoodi*). Should the chains of intergrading subspecies which now connect *artemisiae* with *osgoodi* ever be broken the two forms would be considered distinct species.

That subspecies are incipient species is believed by many zoologists. To constitute a new form in the process of speciation, however, any part of a species may theoretically be split off, either a group of subspecies, a single subspecies, a part of a subspecies, or a local race. To treat subspecies as incipient species, therefore, is likely to produce the misleading conception that only subspecies can differentiate into species (Dice and Blossom, 1937: 118-120).

An important step in speciation is exhibited by the two species *polionotus* and *maniculatus*, both of which are members of the *Peromyscus maniculatus* species group. These two species differ considerably in body size, but are very similar in other characters. In the laboratory they interbreed fairly well, except that the small *polionotus* females when mated with the larger *maniculatus* males usually die at parturition, due to the large size of the hybrid fetuses. *Maniculatus* females mated to *polionotus* males bear the hybrid offspring without difficulty. Both the female and male hybrids are fully fertile (Watson, unpublished). The close relationship of the two species is proved by their interfertility as well as by their similarity in morphological characters, and they

must accordingly have had a common ancestry. Their geographic ranges, however, are now separated by an area hundreds of miles across where neither occurs. The two forms may be presumed not to have interbred for hundreds of generations, but they have not yet diverged sufficiently in their reproductive processes to have become intersterile.

A somewhat similar relationship is exhibited by the two species *leucopus* and *gossypinus*, both of which are members of the *Peromyscus leucopus* species group. These two species also are closely similar in morphological characters, but *gossypinus* is the larger in body size. In the laboratory they are completely interfertile, and both the female and male hybrids are fertile (Dice, 1937b: 1-3). The ranges of the two species are in general different, but they overlap slightly in the Dismal Swamp region of Virginia and in northern Alabama, and more broadly in the lower Mississippi Valley. Where their ranges overlap they occupy in part the same habitats, but there is no evidence for their interbreeding in nature, except for two presumed hybrids reported from Alabama (Dice, 1940: 18-22). It may be assumed that in the differentiation of these two species they were at one time separated in geographic range, and that during that time they diverged sufficiently in psychology so that in nature they do not now find each other attractive as mates (sexual isolation).

When sterility has developed between two closely related species this sterility of course forms a positive barrier to the interchange of hereditary factors and thenceforth the two species are bound to diverge still farther in evolution. One step in the production of sterility between two species is shown by the relationships between *Peromyscus truei* and *P. nasutus*, both of which are members of the *truei* species group. These two species show only small morphological differences. In the laboratory they cross with some difficulty, and the hybrid males are

all sterile, though the hybrid females are fertile (Dice and Liebe, 1937).

The geographic ranges of the species *truei* and *nasutus* overlap broadly in the arid parts of southwestern North America; there are only slight differences in habitat between them; and the two often occur together in the same ecologic communities. In many places, therefore, no geographic nor ecologic barrier prevents their interbreeding. It is probable, however, that there is a difference in mating behavior (sexual isolation) between the two forms, in addition to their partial sterility, for no hybrids have been found in the wild.

In southwestern North America, where the species *truei* and *nasutus* live, there are many isolated mountains and buttes which carry a belt of juniper and Pinyon pine with habitats suitable for these mice. In many of these isolated situations both species occur; in other places only one is found. There are therefore in this region abundant possibilities for temporary geographic isolation of part of a species with the consequent probability of divergence in evolution. *Truei* and *nasutus* are closely related and must certainly have had a common ancestry. Their differentiation most probably began at some past time when a part of the ancestral species became temporarily separated on some isolated desert mountain.

Complete intersterility between two related species constitutes a more complete separation than partial intersterility, and in most cases is probably the next step in speciation. Many of the species of *Peromyscus* have reached the stage of complete intersterility. Notably, no two species of *Peromyscus*, which on the basis of their morphological characters are placed in different species groups or in different subgenera, have ever been successfully hybridized (Dice, 1933: 302-304).

The divergence in hereditary factors between populations isolated from one another should, except for the possible effects of natural selection, theoretically be largely at random. There is, therefore, no reason to

expect that intersterility would be more likely to develop than any other kind of physiological, morphological, or psychological difference. Accordingly, two related but isolated populations might theoretically diverge in morphological characters with little change in psychology or physiology, or they might diverge in psychology or physiology with, for a time, little evident change in morphology. Actually, some *Peromyscus* races and species which appear very dissimilar are fully fertile together, and conversely, some species which superficially appear nearly alike are completely intersterile.

Ultimately, long-continued isolation will theoretically result in the production of intersterility between the several separated populations, in addition to the production of other differences in physiology, in psychology and in morphology. The evolution of intersterility may, however, by the operation of the laws of chance, be long delayed, and races and species which have become widely different in structure or in behavior may still be potentially interfertile.

That sexual isolation is one of the most important factors in the speciation of *Peromyscus* is evident from these studies. Numerous subspecies and species which are fully fertile together under laboratory conditions fail to interbreed in nature, even when they at times occupy the same habitats. If a difference of some sort in the mating psychology of these forms did not exist, they would almost certainly interbreed in nature and thus merge their identities. It is evident, moreover, that once sexual isolation has become established between two forms of common ancestry their divergence is likely to continue until intersterility ensues and separates them irrevocably.

Although geographic isolation is, I believe, an essential first step in the splitting of a species, it is evident that, at least in *Peromyscus*, geographical isolation often does not continue long enough for intersterility to arise. Inherited modifications in behavior leading to sexual iso-

lation between two parts of a species seem more quickly developed. After sexual isolation has arisen, separating two parts of a species, those parts will thenceforth remain distinct, even though their geographic isolation is later broken down. In time, under the protection of this sexual isolation, intersterility between the two daughter forms will be expected to develop, completing their specific differentiation.

Ecological isolation, produced by a divergence in psychology between two parts of a species so that each selects a different sort of habitat, is of some importance in separating forms which otherwise would likely interbreed. However, ecological isolation, when acting alone, seems unlikely ever to be effective completely. In nature there nearly always are some intermediate conditions between the several habitat types, and in these intermediate situations ecological isolation is likely to break down. Ecological isolation, however, undoubtedly has considerable effectiveness in speciation at those places where it is associated with and reinforces sexual isolation.

SUMMARY

The forms differentiated within the genus *Peromyscus* may roughly be classified according to degree of distinction as local races, subspecies or geographic races, species, species groups, and subgenera. Different subgenera and different species groups, so far as they have been tested in this genus, are completely intersterile. Within the *truei* species group two included species are partially intersterile. In two other *Peromyscus* species groups the included species are potentially interfertile, but in nature are separated partly by geographical and partly by psychological (sexual) barriers, so that no interbreeding occurs. Within any given species of *Peromyscus* all the subspecies, so far as they have been tested, are potentially interfertile, but in nature some adjacent subspecies are separated by geographical, by ecological or by sexual barriers. Sexual isolation

seems in this genus to be of especial importance in speciation.

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LEVELS OF DIVERGENCE IN DROSOPHILA SPECIATION¹

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To evaluate the stage of divergence occupied by two organisms in terms of the evolutionary roads over which they have traveled from a common origin and their present place in the space-time continuum of nature is a difficult task. With the knowledge at hand to attempt this for the many complexly interrelated forms in any large group such as that delimited by our present definition of the genus *Drosophila* is to undertake the impossible. However, the venture to summarize some of the relevant data may serve to formulate more clearly the problems involved.

There is, indeed, one fact which favors analysis. This is the rather thoroughly demonstrated principle that at its primary level evolution is built out of discrete and discontinuous blocks, the occurrence of mutations, using this term in the broadest sense, and their distribution among individuals making up populations finite in number. The actual analysis of the evolutionary status of any group of organisms at a given time-level will depend upon a fair sampling of the genetic structure of the populations which make up this group.

For any subdivision of the genus *Drosophila*, other than such isolated populations as occupy half-pint milk bottles the world over, the number of individuals is so large, the distribution of populations so extensive, and the fluctuations in their structure so frequent, that a rough approximation of genetic analysis is all that can be expected. With this understanding of the limitations of analysis, let us examine some of the data.

¹ Read at a joint symposium on "Speciation" of the American Society of Zoologists and the Genetics Society of America, American Association for the Advancement of Science, Columbus, Ohio, December 28, 1939.

DIVERGENCE AT THE GENE MUTATION LEVEL

We shall assume that the primary level of evolution and speciation has to do with the occurrence and frequency distributions of mutations, genic or chromosomal, in populations made up of individuals which are capable of interbreeding freely when the opportunity is offered. It should be understood that the determination of the frequency of gene and chromosomal mutations of the types easily observed establishes a pattern of population structure which may readily be compared with that for mutations not well adapted to ready laboratory analysis, but which may play a more significant evolutionary role than their morphologically more conspicuous fellows.

Over ten years ago Tschetverikov (1927) and the Timoféeffs-Ressovsky (1927) demonstrated by collecting and inbreeding samples of *Drosophila melanogaster* populations that mutant genes were present in heterozygous condition in large numbers. To those who were wont to claim that in some way the laboratory housing and feeding of these animals was conducive to stimulating a sort of germinal upset, these findings must have come as something of a shock.

The most extensive work on the analysis of *Drosophila melanogaster* populations has been done by Dubinin and his colleagues (1934). Wild flies were collected from ten localities in Russia. By the use of special methods, 3,766 autosomes were carefully tested for the presence of lethal and visible mutations. From one locality 84 chromosomes yielded 18 lethals and 26 visibles; from another, 184 chromosomes gave no visibles. Recently Ives (1939) has reported on 151 second chromosomes from a South Amherst population of the same species. He recovered 47 lethal-bearing chromosomes, 18 semi-lethal chromosomes and 12 of the non-lethal bearing chromosomes which carried visibles. Thus Ives found that over 50 per cent. of the chromosomes tested carried mutants.

These data show great differences in the percentage of mutations carried in various populations of *melano-*

gaster. In the investigations by the Timoféeffs in Germany, Tschetverikov and Dubinin and others in Russia, Ives in the United States and Gordon (1936) in England, the *melanogaster* populations studied were breeding outside the natural range of the species. *Melanogaster* is tropical and can not overwinter in the open in any of these geographical areas. The populations are cut down to relatively few individuals overwintering indoors, then attain a peak in late summer and autumn with abundant food and proper humidity and temperature. Year after year small samples of the autumn population fortuitously survive and expand into the next year's maximum. This mechanism provides that in a species with many mutating loci certain local populations will show high frequencies of one or a small group of mutant genes; other populations will show few mutant genes and samples of considerable size may prove to be entirely mutant free. This latter situation seems to have been met with in one Russian population, which may be supposed to have suddenly "bred up" from a small local focus.

Similar studies on lethal gene distribution in populations of *Drosophila pseudoobscura* from the natural breeding range have been made by Sturtevant (1937), and on both lethals and visible mutants for the same species by Dobzhansky and Queal (1938b). In general, the findings are consistent with the view that on the average the effective or minimum breeding population is not cut so low at critical periods in wild populations of *pseudoobscura* in its natural range as in the semi-wild populations of *melanogaster* outside the natural range.

DIVERGENCE AT THE CHROMOSOME INVERSION LEVEL

Of the several ventures into the field of *Drosophila* population analysis, the most extensive in scope and significant in results is the undertaking of Dobzhansky and Sturtevant (1938) to sample populations of *Drosophila pseudoobscura*, races A and B, from many localities throughout their geographical range. The salivary

chromosome analysis of the third chromosome has led to their construction of the probable pattern of relationship between seventeen gene sequences in this chromosome. Through the reasonable assumption that two gene sequences which may be derived one from the other by a single inversion are more closely related than two which differ by more than one inversion, it was possible to trace step by step the evolution of these chromosome sequences. Perhaps the strongest evidence for the validity of the findings was the postulation during the course of the investigation of certain hypothetical alignments which were later actually discovered. The authors of this work do not claim that it gives a complete picture. Certainly it sets a standard for the study of chromosome evolution in animals favored with salivary chromosomes which undergo inversion on an extensive scale.

Recent studies of a similar nature have been reported by Dobzhansky and Socolov (1939) on *Drosophila azteca*, and by Miller (1939) on *Drosophila algonquin*.

Dobzhansky and Queal (1938a, 1938b), Koller (1939) and Dobzhansky (1939a, 1939b) have reported on the proportions of gene rearrangements and lethal mutations in *Drosophila pseudoobscura* occupying separate mountain ranges and canyons in one range near Death Valley, and certain microgeographical areas in Texas and in populations from the highlands of Mexico and Guatemala. The data secured are consistent with the hypothesis that effective breeding populations of small size tend to eliminate some genetic variability and to give rise to high frequency of certain types. Larger effective breeding populations contain more genetic variability with less concentration of specific variants. The findings are in line with the conclusions of Wright (1931) based on mathematical analysis. As a result of these eco-genetic studies we may conclude that local populations, fluctuating year after year and in shorter time intervals in response to conditions of the environment, present the possibility of the fixation and spread of new types here and there over the range of the species.

DIVERGENCE AT THE LEVEL OF INTER-FERTILE RACES
OR SUBSPECIES

Whatever their method of origin, it is of interest to examine the genus for cases of the fixation of an ensemble of hereditary variations. Sturtevant and Dobzhansky (1936) have described *Drosophila affinis iroquois*, differing from typical *affinis* in darker body and leg color. The locality records indicate that *affinis* tends to be distributed through southeastern United States and to be replaced northwards by *iroquois*. *Mahican* was described at the same time as a subspecies of *athabasca*, somewhat lighter in color and with a distribution in the eastern United States as contrasted to the western range of *athabasca*. Subspecific crosses are in both cases fully fertile and the differences between these forms are so slight as to make determination difficult. These cases represent fixation at a primary level of morphological divergence, and the forms appear to differ less from each other than many types which might be extracted from the genetic variability of the average *Drosophila* population.

In 1938 Sturtevant sent to our laboratory stocks of *hydei*-like flies collected in Yucatan, Mexico, by M. Steggerda and called attention to the light thoracic pattern. We have described them (in press) as a new subspecies, *Drosophila hydei yucatanensis*, differing from typical *hydei* in a reduction in size of thoracic pigment spots, larger eyes, a squattier body build and other minor characters. This form breeds true, crosses readily with *hydei*, the hybrids are perfectly fertile and the hybrid salivaries appear to contain no major aberrations. Morphologically, the hybrids are intermediate, but the characters are such that they could be studied only by statistical methods under careful environmental control.

This past summer Harrison Stalker and I trapped two males and two females of a fly near Overton, Ohio. We shall describe it as a new subspecies of *Drosophila macrospina*. *Drosophila macrospina ohioensis*, subspecies

nova, differs from typical *macrospina* (see Stalker and Spencer, 1939) in the following characters. It has a darker eye in contrast to the bright red eye of *macrospina*. The fleck or shadow in the eye of *ohioensis* is less distinct and the eye is smaller than that of *macrospina*. *Ohioensis* has a lighter brown thorax and abdomen, is a little larger on the average and has a less squatty body build. Type *macrospina* was collected in Austin, Texas, in 1935, and sent to us through the courtesy of Dr. Patterson. *Ohioensis* has been taken from near Overton and from a point six miles north. These two subspecies cross readily and the hybrid offspring are quite fertile. Preliminary examination of the hybrid salivaries indicates relatively minor aberrations. Hybrid characters are intermediate to the parent forms. Of the four cases mentioned, this last one probably represents the greatest divergence; however, it would appear that if the two forms of *macrospina* occupied the same habitat range this divergence would rapidly be swamped by intercrossing.

DIVERGENCE AT THE LEVEL OF PARTIAL INTER-SUBSPECIES AND HYBRID STERILITY

Accepting the criterion of inter-group and F_1 hybrid sterility as the soundest basis for seriating different levels of speciation, we may next consider cases in which lowered fertility occurs in inter-group crosses and their hybrids.

Drosophila virilis americana (Spencer, 1937) differs from *virilis virilis* in many morphological and physiological characters, including eye size and color, body- and pupa-case colors, etherization time and pupation habits. When these forms are crossed a few hybrid offspring are produced. The inter-fertility varies greatly with the stock of *virilis* used. Hybrids of both sexes are partially fertile when crossed *inter se* or back to either parent subspecies. In fact, on the average, hybrid fertility is considerably higher than that of the initial cross. Hughes

(1939) has recently published a full account of the cytology of this case. In the metaphase of the ganglion cells of *virilis* there are five pairs of rod-shaped chromosomes and one pair of dots in both sexes. In *americana* the female has two pairs of V-shaped chromosomes, a pair of rods and a pair of dots. In the male there is one pair of V's, one pair of rods, a pair of dots and a single V paired with two rods. The rod-like X chromosome of *virilis* has its homologue in one limb of an *americana* V. The other limb corresponds to a *virilis* autosome. The Y of *americana* is not fused with an autosome; hence the peculiar configuration in the male of *americana*. The salivary chromosomes of both subspecies show five long units and one very short one. In the hybrids the units corresponding to the X, second, fourth, and fifth chromosomes show configurations which indicate that these chromosomes differ by inversions in the two subspecies. Hybrid chromosome five shows very loose pairing.

With cross-sterility as the criterion for determining species, the *virilis* case presents a taxonomic problem. This is augmented by the great difference in cross-fertility between *americana* and the several *virilis* strains tested. In this connection some extremely interesting new material on a group of partially inter-fertile forms in the *virilis* complex is being presented in the genetics demonstrations at these meetings by Patterson and his colleagues (1939).

Recently a new case of hybridization between forms somewhat more distinct than *virilis* and *americana* and producing partially fertile hybrids has been discovered. Last July 27th Stalker and the author collected two new Ohio *Drosophila* in the Killbuck Swamps, Wayne County. One of these has been identified by Sturtevant as *Drosophila lattivittata* Malloch. However, this name is invalid through its prior use for another species. The other form is undescribed. We shall name and describe these forms elsewhere. However, the case is pertinent to

the present discussion and we therefore present a preliminary account.

Upon subsequent collecting, over eighty specimens of the two forms were taken. *Lattivittata* was the more abundant. Both have a beautiful striped and spotted color pattern, and appear to be related to the *quinaria-transversa* complex. The two differ in many morphological characters including size of wing clouds, shape and color of the eye, body color, shape of posterior cross-vein, length and color of pupal horns and external genitalia. Stalker states from tests made in August and September that "both reciprocal crosses are made if large numbers of individuals are used. The hybrid salivaries present a tangled picture, with long regions of loosely paired chromosomes."

The hybrids of *lattivittata* females by males of the other species have eyes intermediate but more like their mothers in shape, and wing cloud and cross-vein shape more like that of the fathers. In the reciprocal cross all these characters more closely resemble those of the mothers. This indicates that eye shape is conditioned either by maternal effects or by sex-linked genes. The hybrids of the cross made in either direction proved partially fertile in mass cultures, though fertility was observably lower than for either parent species. These two forms will probably be described as new species, though they seem to occupy a position as regards hybrid sterility not far removed from the *virilis-americana* complex. They occupy apparently a sharply defined ecological habitat. We have collected many thousands of *Drosophila* in woodland and other areas in Wayne County without taking a single specimen of either species. The discontinuous nature of swamp habitats should offer peculiar advantages for the isolation and development of new variant forms. The problems of ecology so important to an understanding of the factors involved in speciation should be more readily solved in forms known to occupy a definitely delimited habitat. We have al-

ready collected the larvae and pupae of one of these species breeding on the decaying leaf stalks of the broad-leaved arrowhead, *Sagittaria latifolia*.

In the process of speciation both the mechanism and relative time and course of development of incompatibility of the two diverging forms and of the sterility of their F_1 hybrids may quite conceivably differ. Put in its simplest form the question resolves itself into whether F_1 sterility develops prior or subsequent to the inability of the parental forms to produce viable offspring. A third alternative suggested by Sturtevant (1938) is that of initial partial sterility of the hybrids leading to selection of factors favoring the elimination of inter-group crossing. We find in the *virilis* and *lattivittata* cases evidence for the development of a strong incompatibility of the parent forms prior to the development of equally marked hybrid sterility. In the *virilis* case the incompatibility seems to depend largely on psychological factors, less frequent cross-matings and/or less sperm transferred to the female at a mating. We do not suggest that this is a general rule and strongly suspect that the mechanism involved varies from case to case.

DIVERGENCE AT THE LEVEL OF PARTIAL INTER-GROUP AND COMPLETE HYBRID STERILITY IN ONE SEX

The *Drosophila pseudoobscura*, races A and B, and *Drosophila miranda* complex illustrates the stage at which complete hybrid sterility has been attained in one sex, in this case the male. We find it impossible to review adequately in the space allotted the thorough analysis of this material due largely to the researches of Dobzhansky.

In brief, races A and B of *pseudoobscura* were first recognized by Lancefield (1929) as producing sterile male hybrids. The two races are morphologically identical by the usual criteria. Mather and Dobzhansky (1939) have demonstrated slight size differences by statistical methods. Race B, broadly speaking, is northern in its distribution from British Columbia to California, while

race A has a wider range including most of the race B territory and extending eastward into Nebraska and Texas and south into Mexico and Central America. Dobzhansky (1937a) states, "It is solely because of the lack of externally visible distinctions that these two forms are described as races of the same species. By any other criterion they should be considered distinct species." Boche (Dobzhansky, 1937b) has demonstrated partial sexual isolation between race A and race B. Inter-racial crosses occur less readily than intra-racial crosses. Male hybrids in both reciprocal crosses are entirely sterile; female hybrids are partially fertile when crossed to either parent race, and these crosses give rise to a series of types differing in fertility due to various combinations of the parental chromosome complexes. Hybrids show inversions in the salivaries (Tan, 1935), though in view of the prevalence of inversions within races the inter-racial differences are not extreme. Dobzhansky (1937b) has found that the sterility of hybrid males is due to genetic factors carried in all four of the long chromosomes.

Drosophila miranda, first collected by Boche and described by Dobzhansky (1935) is larger than *pseudoobscura*, though morphologically quite similar. This species will cross with either race of *pseudoobscura*. The females from crosses in either direction are normal in appearance and MacKnight (1939) reports that some of them are slightly fertile. The males are completely sterile and differ in both form and viability, depending on the direction of the cross. The chromosome mechanism of *miranda* includes two pairs of X's in the female and a Y and two single X's in the male. Dobzhansky and Tan (1936) have shown through study of the hybrid salivaries of *pseudoobscura* by *miranda* that there are more differences in the arrangement of the chromatin than in any two hybridizing forms thus far mentioned. Most of these involve inversions within chromosome arms.

DIVERGENCE AT THE LEVEL OF COMPLETE HYBRID
STERILITY

The next step in evolutionary divergence may be represented by the two species, *Drosophila melanogaster* and *Drosophila simulans*. Sturtevant (1919) first described *Drosophila simulans*, which has larger eyes, narrower cheek, darker body color and stouter body build than *melanogaster*. The egg filaments of *simulans* are longer, and the male genitalia differ more than in any other hybridizing forms of *Drosophila* known. The cross of *melanogaster* female by *simulans* male is more often successful than the reciprocal. Hybrids of both sexes are entirely sterile. Horton's (1939) study of the hybrid salivaries shows the presence of ten chromosomal rearrangements, one long and four very short inversions, and four minor changes in banding at ends of chromosomes. Natural intra-specific chromosome rearrangements in *melanogaster* are rare. This may account for the minor changes in this case as contrasted to some others.

In the *affinis* group Bauer and Dobzhansky (1936) have reported on crosses of *Drosophila azteca* females to *Drosophila athabasca* males. Hybrids of both sexes are entirely sterile and the hybrid salivaries show marked differences in chromosome alignments. Miller (1939) has secured sterile hybrids from the cross of *algonquin* by *athabasca*.

In addition to the *virilis* complex, I understand that Patterson and his colleagues at the University of Texas are working on other cases involving hybridization in *Drosophila*.

In conclusion, we have attempted to review some of the pertinent data dealing with levels of divergence in *Drosophila* speciation. Starting with variations at the single mutation, genic or chromosomal, stage we have presented cases seriated in terms of inter-crossing forms and increasing hybrid sterility. A survey of these data presents many interesting problems. One of these is the variation in the pattern of evolution from group to

group. In some cases outward morphological changes have remained at a minimum, while major changes in gene realignments and sterility have taken place. In other cases fertility has remained close to normal, but morphological changes in geographically isolated groups have occurred.

Clearly emerges the concept of a vast array of micro- and macro-populations of different *Drosophila* groups, fortuitously fluctuating in size and distribution in time and space in response to changing ecological factors. Academic discussions of natural selection on populations indefinitely large and in breeding equilibrium are obsolete as far as the genus *Drosophila* is concerned. Equally obsolete are purely historical discussions of evolution when the process is taking place all about us.

Finally, with the mass of accumulated cyto-genetic data on *Drosophila melanogaster*, the incomparable tool of salivary chromosome analysis and the ease and speed of culture, *Drosophila* offers unique opportunities for further ventures into the old but always alluring field of the "Origin of Species."

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SPECIATION AS A STAGE IN EVOLUTIONARY DIVERGENCE¹

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Two principal components may be recognized in the process of evolution. First, during the course of evolution the diversity of organisms is increased. Second, evolution involves the development of discontinuity in the living world, since the organisms become segregated into discrete arrays termed races, species, genera, etc. The increase in diversity is accomplished through the production of new genetic variants, mutations in the broad sense of this word, and is counteracted by heredity which tends to preserve the similarity between succeeding generations. The discontinuity is produced by isolation which hampers or prevents the interbreeding of the members of different discrete arrays. On the race level, the geographical isolation is most important; on the species and higher levels, various physiological isolating mechanisms become increasingly more and more effective. Isolation has its antithesis in the mechanism of sexual reproduction and Mendelian recombination which tends to reunite the discrete groups into a single variable mass.

By "speciation" one may understand two different things. One may apply this term solely to the process of the formation of the discrete non-interbreeding arrays, or one may apply it to include the production of variability as well. In the latter case, the term "speciation" becomes synonymous with "evolution." This is more than a question of words; a real problem is here involved which has not been heretofore very clearly stated, much less solved. In a nutshell the problem is whether isolating mechanisms develop as a necessary consequence of the

¹ Read at a joint symposium on "Speciation" of the American Society of Zoologists and the Genetics Society of America, American Association for the Advancement of Science, Columbus, Ohio, December 28, 1939.

accumulation of genetic differences in general, or whether they represent a separate category of genetic changes which appear and become established only under certain special conditions. In discussing this problem two fundamental considerations must be kept in mind. First, the genetic information available shows that the differences between natural races, species, and genera are complex, consisting of numerous genic as well as chromosomal elements. Second, the maintenance of species as discrete entities is possible provided only that their interbreeding be prevented by isolating mechanisms. Unless two or more species are isolated geographically, they must either develop physiological isolation or become fused into a single greatly variable species. In the process of species divergence a complex of genic and chromosomal differences must be formed, and one or more physiological isolating mechanisms must become established.

The traditional view-point, which is implied, if not expressly stated by most taxonomists and geneticists, is that species formation is a graduated and uniform process. The usual view is that if groups of individuals are confined to different territories, and if sufficient time be allowed during which these groups remain separated, they will become distinct first as races, then as species and, finally, as genera or higher categories. This seems to mean that the development of isolating barriers is a necessary corollary to the accumulation of genetic differences. Unless such a simple relation between the genetic differences at large and the appearance of physiological isolation can be demonstrated, the above view is invalid. The alternative seems to be that the development of physiological isolation is a process separate from that which produces the gene complexes responsible for other morphological and physiological differences between the incipient species.²

² Apart from the above alternatives stands the unorthodox opinion of Goldschmidt (1932, 1933) who sees no bridge between riation and speciation. To be sure, Goldschmidt nowhere states that the essence of species differentiation lies in the presence of physiological isolating mechanisms,

The only method of speciation which is at all clearly understood is that by polyploidy. A new species may emerge abruptly following a reduplication of the chromosome complement. It is known in a number of instances that polyploids are crossable only with difficulty to the parental species from which they have been derived, although they are capable of sexual reproduction with their like (Karpechenko, 1928 and others). Hence, in these cases, a physiological barrier arises simultaneously with the formation of a new species. However, species formation by polyploidy is confined to certain groups of organisms, mostly plants. The more general method of speciation is through a gradual accumulation of genic and chromosomal changes. Here the problem of the origin of isolating mechanisms is by no means settled.

It would appear, however, that the accumulation of genetic changes does not necessarily induce isolation. For example, in *Drosophila* strains may be synthesized which differ in more than a dozen genes, but there is not the slightest evidence that the crossability of such strains is lowered. This is not a conclusive proof that strains differing in hundreds of genes would show no limitation of interbreeding, but from all that we know at present this seems very doubtful. So much for genic changes. Structural changes in chromosomes, especially translocations, do produce partial sterility in heterozygotes. Yet, wild species are known which show less alteration of the chromosome structure than can be produced in the laboratory without at the same time inducing isolation. Thus, the so-called "races" of *Drosophila pseudoobscura* (which actually behave as distinct species) produce semi-sterile hybrids when crossed, but differ at most in four inverted sections in their chromosomes. In *D. melanogaster*, heterozygotes for five inversions are fertile, and in *D. pseudoobscura* itself heterozygotes for quintuple in-

but this is the only interpretation that the present writer is able to conceive of his views. Otherwise, the evidence for continuity between races and species is overwhelming.

versions in the third chromosome occur in nature without showing a lowering of the reproductive potential (Dobzhansky, 1937). Although in *D. melanogaster* strains have been constructed artificially which produce no viable offspring when crossed to the original form (Stern, 1936; Kozhevnikov, 1936), the differences which exist between wild species have not been copied.

The problem may be approached from another angle, namely, by studying the genetic basis of the isolating mechanisms actually encountered in nature. Such studies might reveal whether a relation exists between the genetic factors producing isolation and the factors causing morphological and physiological differences between species. The sterility of hybrids between the "races" of *Drosophila pseudoobscura* proved to be due to numerous complementary genes scattered apparently in all the chromosomes (Dobzhansky, 1937b and other publications). Furthermore, within each race genetic variants were encountered which were similar to, or identical with, the genes composing the sterility barrier between the races. Thus, one can visualize the building up from the genetic elements available within a species of the sterility barrier which separates species. The point which interests us most at present remains, however, not settled: It is unknown whether the "sterility genes" produce, by manifold effects, the morphological and physiological differences between the races, or whether separate groups of "morphological" and "physiological" genes on the one hand and of "sterility" genes on the other are involved. The latter alternative seems, however, more probable.

As indicated above, the view which seems most likely to be correct on the basis of the genetic information available at present is that the origin of isolation is a process separate from that of the origin of other species differences. An attempt can now be made to outline a theory that would help to visualize the interrelations between these two processes. This theory starts with the

premise that each species, genus and probably each geographical race is an adaptive complex which fits into an ecological niche somewhat distinct from those occupied by other species, genera and races. The adaptive value of such a complex is determined not by a single or a few genes, but is a property of the genotype as a whole. Furthermore, the adaptive complex is attuned to its environment only so long as its historically evolved pattern remains, within limits, intact. It is true that interbreeding of different adaptive complexes may sometimes result in emergence of new genotypes which fit into unoccupied or sparsely settled ecological niches—hence the evolutionary role of hybridization. Nevertheless, hybridization usually leads to the formation of disharmonious recombinations.

Considerations such as these have prompted some writers (Dobzhansky, 1937a, b; Sturtevant, 1938; *cf.* Fisher, 1930) to assume that occurrence of hybridization between races and species constitutes a challenge to which they may respond by developing or strengthening isolating mechanisms that would make hybridization difficult or impossible. Where hybridization jeopardizes the integrity of two or more adaptive complexes, genetic factors which would decrease the frequency or prevent the interbreeding would thereby acquire a positive selective value, even though these factors by themselves might be neutral. Race formation is essentially the development of genetic patterns which are adapted to a definite environment. Speciation is a process resulting in fixation of these patterns through the development of physiological isolating mechanisms. Clearly, raiation and speciation should not be conceived of as entirely independent processes, but the development of physiological isolating mechanisms must nevertheless be supposed to intervene only after the divergence of the adaptive complexes had been initiated. If races are to become species, isolating mechanisms must arise when the distinct adaptive complexes are exposed to the risk of disintegration due to

interbreeding. It may be of interest to discuss some evidence that appears to support or raise obstacles to this theory.

Races of the same species are, as a rule, confined to different territories. Not infrequently races merge into one another; in passing from the territory of one race to that of another, geographical gradients, or to use the term recently proposed by Huxley (1939), "clines," in the differentiating characters are encountered. The clines are seldom perfectly gradual; more commonly the cline is much steeper in a certain zone than in others. The zone of the steep cline is the geographical boundary between the races. It is in these zones that the exchange of the elements of the gene complexes of the races is most frequent, and hence the formation of isolating mechanisms of any kind is most easily accomplished. The traditional belief seems to be that a race first becomes a species at the center of its distribution; the view here advanced implies that speciation is initiated primarily at the boundaries between races.

The lowering of the adaptive value of the recombination products from interspecific crosses is attested both by experimental data and by observations in nature. An excellent case of this kind has been described by Meise (1928). The distribution areas of the two crows, *Corvus corone* and *Corvus cornix*, are apposed to each other along a line some 3,000 kilometers long winding across Europe and Asia. A narrow zone on either side of this line is populated by obvious hybrids, and yet the interbreeding products do not seem to diffuse toward the main bodies of the parental species. Meise presents convincing evidence to show that *C. corone* and *C. cornix* were separated during the Ice Age, and that their distributions converged in the post-Glacial time. Nevertheless, the hybridization zone seems to be even narrower where the contact between the species had been established long ago than it is where the contact is more recent. This is an example of a situation where physio-

logical isolation has not yet developed despite the occurrence of interbreeding, and despite the obvious inferiority of the recombination products. It can hardly be doubted that any genes which prevent or diminish the frequency of hybridization would have a positive selective value, at least in the boundary zone. We are confronted here with that critical stage of speciation where the classification of two forms as races or as species is arbitrary.

An interesting regularity has been revealed by the study of the geographical distribution of the factors that modify the extent of the sexual isolation between *Drosophila pseudoobscura* and *Drosophila miranda*. The area of *D. miranda* is broken into two parts, one in the Puget Sound region and the other in the central Sierra Nevada. Both parts are included in the distribution of *D. pseudoobscura* which extends from British Columbia to Guatemala. As a rule the strains of *D. pseudoobscura* from the Puget Sound region show the strongest, and those from places remote from Puget Sound, an intermediate or weak isolation from *D. miranda*. Moreover, the genetic factors which modify the sexual isolation between *D. pseudoobscura* and the northern race of *D. miranda* are distinct from those which influence the behavior of *D. pseudoobscura vis-à-vis* the southern race of *D. miranda*. It is evident that the genes increasing the isolation between *D. pseudoobscura* and the Puget Sound *D. miranda* are of highest selective value in those populations of the former species which reside in or near the area where the latter species also occurs (Dobzhansky and Koller, 1938).

That isolating mechanisms may develop in the regions where the interbreeding of related species is taking place is plausible enough. One of the difficulties with the theory lies in explaining how the gene complexes responsible for the isolating mechanisms come finally to permeate the whole bodies of these species. What selective value have the isolating mechanisms for those parts of the species which are not, and never were, exposed to the danger of interbreeding with other species? A possible

answer to this question is that if certain genes are favorable in a part of the species area and neutral elsewhere, they will eventually diffuse throughout the species by migration. The diffusion will certainly require time, and hence one may expect the degree of isolation between two species to depend upon the geographic origin of their representatives. As stated above, this is the case in *Drosophila pseudoobscura* and *D. miranda*. Here is an almost totally unexplored field for future studies. It must also be taken into account that "isolating genes" must be considered as an integral part of the species genotype as a whole. The introduction of new elements in the form of the originally neutral "isolating genes" may eventually cause a general reconstruction of the species genotype so that these genes may acquire the role of an essential part of the system, and hence an adaptive value outside of their original province of begetting isolation.

Another difficulty is with species isolated on oceanic islands or in similar situations. If the geographical barriers between races are secure enough, the precondition for the development of physiological isolation is absent. Yet, insular speciation is a classic example of speciation in general. Precisely how serious is this difficulty is not clear, since experimental data on the presence or absence of physiological isolation between insular species seem to be lacking; this is another fertile field for future work. Cases are known in which a species having developed on an island subsequently migrates to the mainland or to another island having a related species of its own, and no interbreeding results. It seems reasonable to suppose that immigration had occurred repeatedly, and that the migrants have become established only after the development of physiological isolation due to the previous intrusions. On the other hand, it is possible that certain forms of physiological isolation may occasionally arise as by-products of the adaptation to the environment. Thus, adaptation to different food plants

or soils may engender ecological isolation; adaptation to different climates may lead to a divergence of the breeding seasons (temporal isolation); physiological changes of various kinds may affect the sexual behavior, recognition marks, or smells, and give rise to sexual isolation. The basic problem which remains to be settled is how frequently and to what extent can the isolating mechanisms be regarded adaptational by-products arising without the intervention of the special selective processes postulated above. Only experimental data could elucidate the situation further.

A very remarkable fact long known to taxonomists is that species in different groups, even among the sexually reproducing organisms, appear to be unlike entities. Thus, the morphological gaps between related species may be large or (as in *Drosophila*) very small; some species are variable and highly differentiated, others are clear-cut and uniform; the bar to interbreeding may be ecological, or due to sexual isolation, or to the structure of reproductive organs, or to hybrid sterility or to a combination of these and other causes. The unlikeness of species has led some biologists to abandon the search for properties common to all species—an example of faulty thinking. A dog, a bat, and a whale certainly do not look very similar, and yet zoologists recognize them to be members of the same class—mammals. Isn't it a task of science to detect fundamental similarities concealed by apparent unlikeness? A fundamental common property of species is the presence of isolating mechanisms. The very fact that isolating mechanisms are as diversified as they are is strong evidence for the prevention of interbreeding being an essential characteristic of the process of speciation. The precise means whereby the interbreeding is eliminated are immaterial so long as the exchange of genes is precluded. Any gene that raises an effective barrier to the mingling of incipient species is adaptively valuable, and hence may become the basis of speciation.

SUMMARY

By speciation is meant the fixation of discontinuity among organisms. Discontinuity is maintained by isolating mechanisms that prevent the interbreeding of carriers of different adaptive complexes of genes. A theory is suggested according to which the development of isolating mechanisms follows, rather than accompanies, that of the adaptive complexes themselves. The development of physiological isolation takes place principally along the geographical boundaries separating the distribution areas of the incipient species. Some evidence for and against this theory is discussed.

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OBSERVATIONS ON THE ECOLOGY AND NATURAL HISTORY OF ANURA

I. HABITS, HABITAT AND BREEDING OF *BUFO COGNATUS* SAY¹

ARTHUR N. BRAGG

INTRODUCTION

It has been some time since an adequate study has been made concerning the natural history of any single species of North American toad. Miller (1909) published an extensive account of the natural history of the American toad (*Bufo americanus americanus* Holbrook) based upon observations made in central Massachusetts. Some years later, Wright (1914) studied the same form in central New York. The life-histories of many of the eastern species have also been given by Wright (1931). Except for studies on feeding habits, short articles and incidental notes, these are the latest papers which have come to my notice.

The natural history of the western species of toads is quite inadequately understood. After the settlement of the central and western plains, biological workers in these regions quite naturally concerned themselves first with biological surveys and with the attendant problems of taxonomy and distribution. Such studies are still necessary and many are in progress, especially in the more sparsely settled areas, but it seems now that sufficient knowledge has become established for some studies to be directed more specifically toward an understanding of the lives of the individual animals themselves as they occur in their natural habitats.

It might be supposed by those unfamiliar with the environmental conditions encountered by animals inhabiting the plains that Miller's and Wright's papers on the American toad would give an adequate basis for understanding the habits of American toads in general. Indeed, Over

¹ Contributions from the Zoological Laboratory of the University of Oklahoma, No. 197.

(1923), speaking of *Bufo cognatus* in North Dakota, remarks, "Its . . . life habits are the same as those of the common toad" (*B. a. americanus*). It is one purpose of this paper to show that this is far from the case, at least in central Oklahoma. The adaptations to specific environments in the drier Southwest offer much of interest and value in comparison with those of the moister and cooler eastern section of the United States. In ecological terms, it is to be expected that habits which adjust a given species to complexes of environmental conditions in the grasslands biome will be different, both qualitatively and quantitatively, from those in the climax beech-maple forests of the East. These ideas were hinted in an earlier paper (Bragg, 1936) in which it was shown that the distribution of species in the three principal American families of Anura (*i.e.*, Ranidae, Bufonidae and Hylidae) follows closely the major climatic regions of the country.

Some examples of the differences in breeding habits of Anura in the East and of those in the western plains will make this point clear. *Bufo a. americanus* breeds fairly early in the spring with the crest of this activity about April 30 (Wright and Wright, 1933). The two common toads (*Bufo cognatus* and *B. woodhousii woodhousii*) of central Oklahoma have a breeding season extending well into the summer, and both of these usually breed only after rain. In any one year and at any given place, neither of these toads will breed in any numbers if rain does not come, even though there be plenty of water. Nor are these differences limited to toads. *Rana pipiens pipiens*, the common leopard frog of the East, begins breeding when the water temperature reaches 43-45 degrees F., with the crest of the period reached at 50-65 degrees F. (Wright, 1920). These temperatures are usually attained in New England at about April 1, but eggs of this species have been collected in Maryland as early as the middle of February in some years. In contrast to this, *Rana sphenoccephala*, the southern leopard frog, which is the ecological equivalent of *R. p. pipiens*,

breeds about Norman, Oklahoma, at any time from late winter to late summer. I have seen them breeding or have collected their eggs in almost every month from February to August and at air temperatures ranging from about 40 degrees F. to over 100 degrees F.

Similar contrasts might be made between many ecological equivalents from these two general regions of the country, but nowhere are they more strikingly shown than among the semi-terrestrial Amphibia—and, of these, the toads (*Bufonidae*) are of special interest.

For several years, I have been observing the habits of the Amphibia, more especially of the toads, in central Oklahoma, principally during the breeding season. In the course of this study, which necessarily has been largely seasonal, one species has been emphasized, namely, the Great Plains toad, *Bufo cognatus* Say. It is the purpose of this paper to summarize the existing knowledge of the natural history and ecological relationships of this toad and to report observations made upon it mostly during the spring and fall of 1938. I know these toads only as they occur in the vicinity of Norman, Oklahoma, and all statements made which are unsupported by reference to the literature are to be interpreted as applying specifically to this area. The study is based upon extensive observations in the field at all hours of the day except between 2:00 and 8:00 A.M., supplemented by studies of the eggs, the embryos, the process of hatching and the process of metamorphosis made in the laboratory in conjunction with the studies in the field. The view-point has been that field observations are necessary if one is to understand the lives of the toads in nature, but that certain phases of the life history can best be observed in the laboratory, where equipment can be more easily used and measurements more conveniently made.

ACKNOWLEDGMENTS

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HISTORY OF *BUFO COGNATUS*, ITS RANGE AND HABITAT

Bufo cognatus was named and described briefly by the early American naturalist, Thomas Say, who found it on the Arkansas River in what is now Prowers County, southeastern Colorado. (For the original description, see Long, 1823.) Other early naturalists found it on the Plains (Holbrook, 1842; Baird, 1859; Yarrow, 1875; and others) and in later years scattered records of its occurrence have come from most of the states of the Great Plains (see Fig. 1).

Its general distribution includes the following states: Minnesota, North Dakota, South Dakota, Montana, Wyoming, Nebraska, Kansas, Colorado, Utah, Oklahoma, Texas, New Mexico, Arizona and California. It has also been taken in Mexico south of the Texas border. However, its range does not include all portions of each of these states. Strecker's papers on several of the counties of eastern Texas do not record this species. No authentic record is known from Arkansas (Black and Dellinger, 1938) and it does not occur in eastern Kansas, so far as known (Smith, 1934).

The first record from Oklahoma which I have found is that of Baird and Girard (in Marcy and McClellan, 1854) who figure a specimen collected in southern Oklahoma just east of the Wichita Mountains. So far as now known, the species occurs rarely if at all in eastern Oklahoma. It was

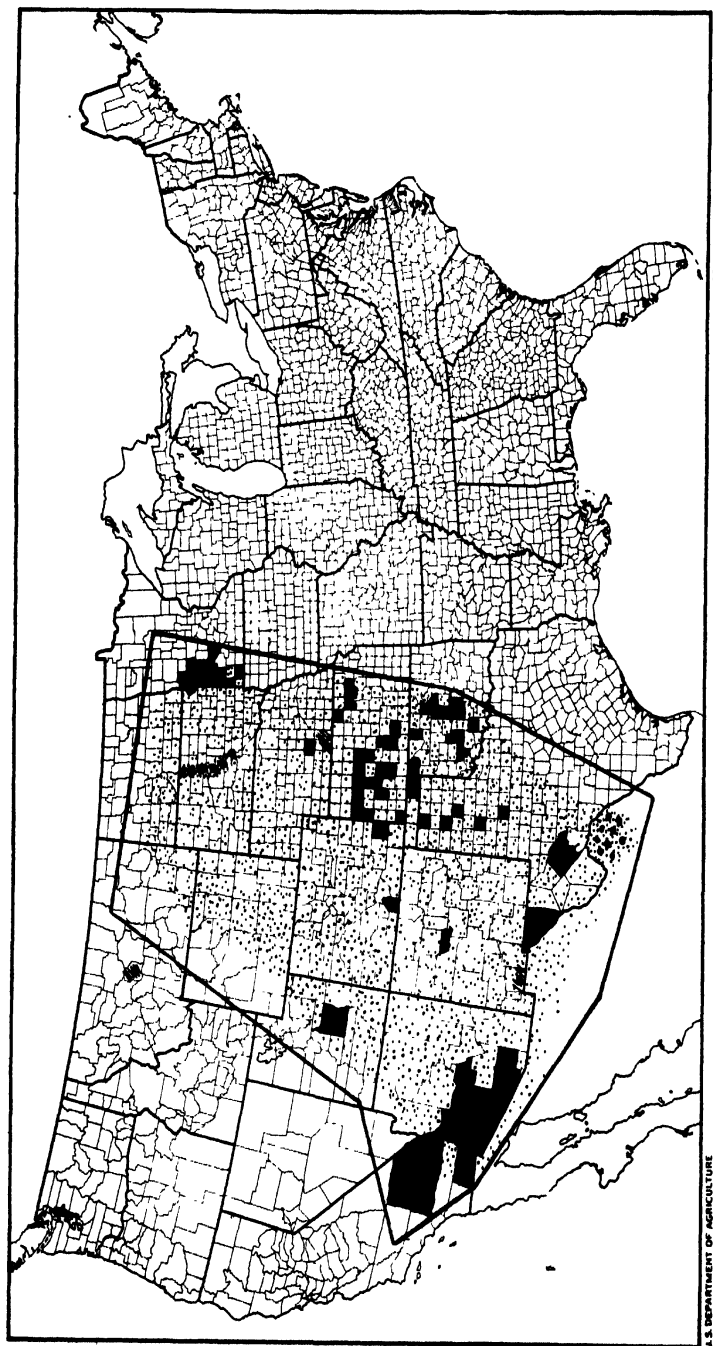


FIG. 1. General distribution of *Bufo cognatus*. Solid black indicates county records; large dots, records in the general area shown; cross-hatching, records considered doubtful, either as to fact or as to county; stippling, general range; black line bordering the whole, the probable area in which *B. cognatus* is likely to occur in proper habitats. All records outside Oklahoma were compiled from the literature cited; in Oklahoma, the specimens in the University of Oklahoma Museum of Zoology and personal observations supplemented the literature.

NEG. 87588 BUREAU OF AGRICULTURAL ECONOMICS

U.S. DEPARTMENT OF AGRICULTURE

not collected by Trowbridge (1937, 1937a) in the southeastern part of the state and had not been reported east of Pottawatomie County a few years ago (Ortenburger, 1927). This is of interest because Pottawatomie County joins Cleveland County on the east, where all my observations have been made and where this toad is extremely abundant. Since the grasslands biome is interrupted by an oak-hickory associates in eastern Cleveland County, this definite break in the distribution to the eastward is to be expected. *Bufo cognatus* occurs in great abundance in central Oklahoma but more sparingly in the western portion of the state.

The northern limits of the species seem to be southwestern Minnesota (Breckenridge, 1938), the southwestern limits, the waters entering the Salton Sea in the Imperial Valley, California (Myers, 1930). The recent record from Minnesota suggests the presence of this form in northwestern Iowa at the present time, but Ruthven (1910) did not find it there. I have found few definite records from New Mexico, although Bailey (1913) lists this species as a characteristic inhabitant of his lower and upper Sonoran life-zones, which comprise most of the state. Cary (1917) lists it in his Sonoran life-zone in Wyoming but not in the transition zone of this state.

Within its general range, *Bufo cognatus* varies considerably in the number of individuals present in various fairly limited regions. For example, Ortenburger and Freeman (1930) did not collect this species in western Oklahoma, although they found *B. woodhousii woodhousii* to be fairly common. Similarly, Linsdale (1938) did not take it in his extensive survey of a limited portion of the Great Basin, Nevada. Ortenburger and Ortenburger (1926) saw it but rarely in Pima County, Arizona.

These observations are explained in part by what is known about the habitat of this toad. Wright and Wright (1933) summarize this as follows: "grazing lands or agricultural lands of the Great Plains, along irrigating ditches, flood plains of streams and overflow bottom lands."

Ruthven (1907) found these toads common "at dusk about irrigation ditches, but not elsewhere" in a region climatically deficient in rainfall (Arizona). Strecker (1910) found them extremely abundant in low places on the plains of northwestern Texas during wet seasons. Ruthven (1932) collected this species on the flood plain of the Green River, eastern Utah. In contrast, Bragg (1937) reported this species as mostly confined to the *higher* portions of the prairies and seldom in the immediate vicinity of inhabited portions of the city of Norman, Oklahoma (see Fig. 2

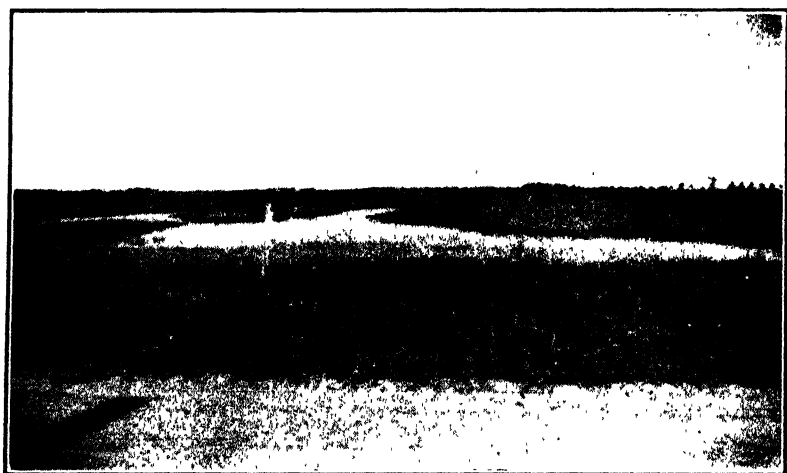


FIG. 2. A typical habitat of *Bufo cognatus* just south of Norman, Oklahoma. The field in the background has a large population of this species. In 1938, the toads bred in the flooded field shown in the middle distance.

for a typical habitat in this region). They have never been taken, so far as I can determine, on the extensive flood plain of the South Canadian River near Norman, even when present in enormous numbers on the prairie just above it during the breeding season. These observations are confirmed by Dr. Charles Smith, who tells me that the toads of a river-bottom farm in northern Logan County, Oklahoma, are all *B. w. woodhousii*, although, above it, on higher ground, *B. cognatus* is abundant. Brennan (1937) similarly found them only in the prairie habitat in central Kansas.

These differences in the observations of the various field workers seem to indicate that *B. cognatus* occupies different habitats in the different portions of its range. Since, in Oklahoma, these toads are quite susceptible to the influence of moisture, it is probable that the habitat occupied by them in any one region is largely conditioned by this factor. They seem to like it neither too wet nor too dry. They constitute the desert form in the Imperial Valley (Myers, 1930) but I can not agree with Myers that "*B. cognatus* is strictly a desert animal throughout its range. . . ."

From the bulk of the evidence available, it may be concluded that *Bufo cognatus* is primarily a toad of the grasslands biome which is able to extend its range into deserts of the Sonoran zone in limited numbers along irrigation ditches and similar low-lying areas where sufficient moisture is available. Under climatic conditions resulting in a mixed grass prairie, however, it tends to avoid the lower areas, thus automatically becoming excluded from woodlands and the flood plains of streams.

TAXONOMY

The taxonomic relationships of *Bufo cognatus* have presented little difficulty. Some of the early workers considered this toad a subspecies of the old eastern species *lentiginosus* and, hence, refer to it as *B. lentiginosus cognatus* (e.g., Yarrow, 1875; Cragin, 1881), but all modern authorities recognize it as specifically distinct. Cope (1875) reinterpreted the type specimen of *B. dipternus* Cope as a young individual of *B. cognatus*. Camp (1915) described a new form from Santa Paula, Calif., as a subspecies, *Bufo cognatus californicus*, as a result of which Say's toad automatically became *B. c. cognatus* (Say) and was so called by many herpetologists (e.g., Storer, 1925; Ortenburger, 1927; Gloyd, 1929; Tanner, 1931). Since Myers (1930) clearly demonstrated that Camp's toad is specifically distinct from *B. cognatus* Say, the trinomial designation has seldom been used (Wright and Wright, 1933;

Smith, 1934). Hence, nearly all are now agreed that the correct name is the original one given it over a century ago, *Bufo cognatus* Say, with *B. dipternus* Cope the only synonym.

DESCRIPTION OF ADULTS

The recognition of *B. cognatus* is relatively easy (see Figs. 3 and 8). It is a medium-sized to large toad, the



FIG. 3. Adults of *Bufo cognatus*, male on the right, female on the left.

females slightly exceeding the males in size. Wright and Wright (1933) give 47–95 mm and 60–99 mm as the lengths of the male and female adults, respectively. Smith (1934) records a female 114 mm in length. Boring and Liu (1934) indicate that some individuals may reach 115 mm. Thirty-five calling males, collected at Norman, Oklahoma, on March 28, 1938, ranged in length (after preservation in formalin) from 72–90 mm, with an average a small fraction above 80 mm. These figures indicate a size slightly larger than the American toad (54–110 mm) but smaller than *B. w. woodhousii* (56–118 mm) according to Wright and Wright (1933).

Its color is variable, but there is commonly a greenish cast to the dorsal and lateral portions; and when these are

not green, they are almost always gray or brown. The back is marked with large dark spots, greenish, dark brown or almost black and somewhat irregular in outline. These are usually arranged approximately in pairs, one of each pair on either side of a faint, light-colored mid-dorsal stripe. Sometimes the stripe may be absent or very faint. Occasionally one finds a specimen which has only a few spots, in which case they are small and scattered over the back (see Wright and Wright, 1933, Pl. XV, Fig. 3, for a typical example). Myers (1930) found that specimens from the region of the Pecos River, Texas, varied more in color and markings than is usual. On the sides are smaller spots, often broken up into vermiculations. The ventral surface is uniformly light, except that the vocal sac of the male forms a black, sooty-colored apron on the throat. This secondary sex character is very useful in distinguishing the sexes. On a few four-month-old females, I have observed a dark pectoral spot such as often occurs in *B. w. woodhousii* (Smith, 1934) and in *B. w. fowleri* (Wright and Wright, 1933), although some others examined lacked this character. It is interesting that I have never noticed this spot on adults, although I have handled hundreds of them.

One of the most characteristic and obvious features of the species is the presence of an osseous boss, just anterior to the eyes on the dorsal surface, from which two cranial crests extend posteriorly. These crests diverge from the boss in such manner that they commonly form a distinct V-shaped structure between the eyes, very conspicuous on old adults, and noticeably present on individuals just less than two inches long. Cope (1875) found that his young specimens (one and one half inches in length) lacked the crests, and I have specimens of this length which also have not yet developed them, although others but slightly larger show them faintly. Only occasionally are these crests less distinct than described. Therefore, they serve as an extremely useful character in the recognition of the species.

The parotoid glands are prominent, set wide apart, ovoid in shape, and extend obliquely postero-laterad from just posterior to the eyes. The foot has two metatarsal tuber-

cles, each with a cutting edge, one of them larger than the other. These are used for burrowing.

GENERAL HABITS

This, like most American toads, tends to be nocturnal in habit. However, Strecker (1910) and Bragg (1937) have occasionally found them active during daylight. This is especially noticeable during moist weather and during the height of the breeding season. At times of prolonged drouth, especially when temperatures are high, these toads do not appear at all even during the night; at least, repeated search during such times has never produced a specimen in areas where they are known to be very abundant under other conditions. A light shower, however, will often bring them out in numbers.

When not active, these toads rest below the surface of the ground in burrows which they dig with the tubercles of the hind feet, the animal, in all probability, backing into the ground much as *Scaphiopus* is known to do and eventually becoming covered completely. The burrows have been little studied, for they are difficult to locate once the toad is below the surface. I have found but few during several years of observation; in one, the toad was below the heated portion of the upper crust and it seems probable that most burrows are deep enough for the animal to avoid excessive heat and to have moisture available. During breeding (which never occurs except when the ground is moist) the burrows are often quite shallow, mere depressions just fitting the body of the toad (Bragg, 1937). Whereas several toads may be found in such shallow burrows on a day succeeding a night of active breeding, on some occasions I have found none at all so occupied even after considerable search, in regions where I have found them before. Also, after a night of active breeding, adults which have entered a pool during darkness may spend the day in the pool. I have noted on several occasions spent or unspent females, males and mated pairs, separately or all together in the same pool. On the whole, it seems likely

that few individuals spent the day after breeding in either the pools or in shallow burrows as above described, for at several times when hundreds of toads have been seen in and about a series of buffalo wallows on an evening, none at all or but two or three have been found during the next day in either situation.

BREEDING

The breeding places of *Bufo cognatus* are often quite restricted. According to Strecker (1910), they bred in temporary pools and small streams in Texas. In central Oklahoma, however, I have found them breeding only in temporary rain-formed pools, specially in so-called buffalo wallows.

These wallows, which occur abundantly in pastures, are shallow depressions of various shapes but often approaching a circle in outline. They were formed in earlier days by the rolling of bison, although cattle now often form similar wallows (Barkley and Smith, 1934). Such wallows are being formed at the present time in the buffalo pasture in the Wichita Mountains in southern Oklahoma. Some of the smaller ones are but a few feet in diameter, but larger ones may measure twenty or more feet. Two of them, measured on May 26, 1938, near Norman, Oklahoma, had the following dimensions:

(1)

North-south diameter	23	feet.
East-west diameter	20.5	"
Depth	5.5 to 9.0	inches (average, about 7 in.).

(2)

North-south diameter	9.5	feet.
East-west diameter	9.25	"
Depth (maximum possible)	10.0	inches (in center).
Depth of water at this time	8.5	" " "

The buffalo wallows are available to toads only in areas which have not been cultivated, for it is obvious that plowing of the land will completely destroy them. Under primitive conditions, they must have been much more numerous than at the present time and probably form the

natural breeding sites for the Great Plains toad in central Oklahoma. Typical buffalo wallows are shown in Fig. 4.

At present, the toads inhabiting cultivated areas often



FIG. 4. Buffalo wallows just northwest of Norman, Oklahoma (station 1). *Bufo cognatus* bred extensively here in the spring of 1938.

use flooded fields for breeding (Fig 5). I have found them in fields planted to rye or wheat but always only in the shallower portions of the pools. But once have I found a *B. cognatus* in deep water in a grain field, and this was a male actively swimming toward a chorus of this species in shallow water near the bank.

Bufo cognatus will also breed in shallow water along the edges of larger temporary pools, not located in cultivated fields; but only if the water is not muddy. One such pool (Fig. 6), just northwest of Norman, has been formed by three embankments where a road crosses an interurban track and a railroad running parallel to each other. The embankment for the road constitutes a dam to the south and the water settles above it, being held to the east and west by the embankments of the two tracks. The pool so formed is quite extensive (172 by 52 ft.) especially after heavy rains in the spring. It is about two feet in depth



FIG. 5. Flooded ditch with a flooded field in the background. Picture taken during the height of the breeding season, 1938, just south of Norman, Oklahoma. This site has been watched closely since 1934 by Mr. A. H. Trowbridge, by myself or by us both and no evidence has been found that *B. cognatus* ever uses the ditch for breeding, although the field was used extensively in 1938.

in the southwest corner (varying, of course, with the amount of rain) and for some distance along the west side.



FIG. 6. The pool at station 4 looking northwest. Great numbers of *Bufo cognatus* eggs were laid in the northern end of this pool in 1938, but no larvae nor emerging young were found in the deeper water in the foreground.

To the east and north, it becomes progressively more shallow except at the south end, the east side of which is but slightly less deep than the west.

Bufo cognatus used this pool extensively during the spring of 1938 for breeding, but only the shallower portions of it. Even fully grown tadpoles avoided the deeper water, for I took relatively few from the southern portions of the pool at times when they were very abundant in the shallower portions. Also, at metamorphosis, thousands of young emerged from the water at the northern end and along the northern half of the east and west sides, but not a single specimen was ever found emerging from the deeper water near the southern bank.

In the region studied, *B. cognatus* rarely lays its eggs in muddy water, although I have occasionally found the tadpoles in fairly roily pools in wheat fields. I have never known them to breed in the relatively permanent pools, known as "tanks," in pastures even when present in large numbers about buffalo wallows close by. They do not breed in these even after spring rains which have been insufficient to fill the buffalo wallows, although the other common bufonid in this region, *B. w. woodhousii*, does so. Muddy ditches attract males, for I have seen dozens of calling males about them. However, I have never found eggs or tadpoles in ditches nor any evidence of their having been there. Once I found a mated pair in a ditch, but repeated search subsequently showed no tadpoles of this species here. I suspect that the depth of water is usually too great in ditches for breeding to occur.

The breeding habits of this species are most interesting. According to Wright and Wright (1933), the breeding season extends from April to September (May to July in the northern portions of the range) and is dependent upon rainfall. In central Oklahoma, I have found them breeding from March 29 to June 17, dependent not only upon rainfall but also upon temperature. This is shown by the following observations made during the spring of 1938.

On February 27, pools were full of water from recent

snow and rain. As might be expected, no toads were breeding. On March 4, the air temperature reached 78° F. during the day and was 64° F. at 7:15 P.M. The pools were full of water, but no toads appeared about them up to 10:30 P.M., at which time the temperature was 60° F. Some of the other *Amphibia* were breeding, but none of the *bufonids* of the region were included. For some time thereafter, daily temperatures were variable, often reaching or exceeding 70° F., but the toads did not breed. Meanwhile, the water in the pools nearly all disappeared through drainage and evaporation. On March 26, over two inches of rain fell, extensively flooding fields and filling pools. That evening the temperature was 9° C. No toads were out. The next day was marked by intermittent showers and a slightly rising temperature. *Scaphiopus* males were calling in some numbers at 9:15 P.M., as was also a species of *Pseudacris*. One male of *Bufo cognatus* was found beside a ditch. The temperature at this time was just less than 12° C., and the animal was slow in its movements as though stiff with cold. This was the only *Bufo* seen. The next day, March 28, it rained hard all the forenoon but ceased some time after noon; over four inches of rain fell. Beginning in the early morning and lasting throughout the day, *Anura* of several species could be heard calling. That evening, *Bufo cognatus* were out in large numbers, calling from flooded fields, ditches and buffalo wallows. Over four hundred were collected just west of Norman by a party from the university in less than one and one half hours; there must have been thousands of individuals within the township. The air temperature at 10:20 P.M., taken in the field just west of Norman, was 12+° C. At 1:00 A.M. it was just less than 14° C. It is significant that six people, all collecting or studying toads in the field about Norman that night, all found females to be quite rare. I saw but three, none of which was in the water with the males. Dr. J. Teague Self found a single mated pair, the female hopping between buffalo wallows carrying the male on her back. No eggs were in the pools at

4 P.M. and later the following day, but that evening occurred the greatest chorus of amphibian voices heard hereabouts since the spring of 1935. Thousands of *B. cognatus* were in and about all favorable breeding sites, and mated pairs were found as early as 10:30 P.M. Eggs were numerous in several pools the next morning and, in some pools, eggs were still being laid during the afternoon. The temperature during the height of this breeding congress (11:30 P.M.) was 18.5° C.

From these observations it appears (1) that *B. cognatus* breeds only after rain; presence of sufficient water is not enough in itself to stimulate the toads to breed. This is to be emphasized, inasmuch as Linsdale (1938) found *Scaphiopus hammondi*, a species which also is commonly supposed to breed only after rain, breeding in fields flooded by mountain streams in Nevada at a place where no rain had fallen for some time. (2) *B. cognatus* breeds after rain only if the temperature is not too low. Twelve degrees Centigrade is apparently the critical temperature, with males slightly more sensitive than females in their response at or near this temperature. As a comparison, it is perhaps well to mention that very few *B. w. woodhousii* appeared during great abundance of the breeding of *B. cognatus* on March 29 and that all that were seen were males. Males of *Scaphiopus hammondi* were also much more abundant on both March 28 and 29 than were females. The critical temperatures for these species, therefore, while close, is not the same for all; that of *S. hammondi* is probably somewhat below 12° C., whereas that of *B. w. woodhousii* is above it.

On May 7, an observation was made which shows that *B. cognatus* will continue to call even if the air temperature falls considerably below that critical for their emergence for breeding, provided that they are already in congress when the temperature falls. A heavy shower fell during the night of May 6. The next afternoon (4:15 P.M.) a large and lively mixed chorus of *B. cognatus* and *B. w. woodhousii* was observed in a flooded field. The latter

toads were not numerous and called but intermittently. The air temperature near the site was 16° C. and the water temperature in an adjoining ditch was 20° C. Late in the afternoon, the weather suddenly turned cold. At 11:00 P.M. the air temperature was 5.5° C. in an unprotected place in the city and some time later it was 8° C. close to where the toads were calling. Water in the ditch continuous with the field had a temperature of 14° C. *B. cognatus* were still calling here in some numbers. The calls appeared distinctly lower in pitch than during the afternoon and the tempo was much slower. I recorded in my notes of this incident that the calls reminded me of an alarm clock which was just running down. The air temperature was considerably below that found to be critical for the appearance for breeding after rain, but the water temperature was probably not much below 12° C., since it was 14° C. in the deeper water of the adjoining ditch. A pair of toads seen laying in a buffalo wallow at 5:00 P.M., just before the temperature started to fall, finished producing eggs and left the pool despite the lowered temperature. Several other incidents of this type have been observed more casually.

From the observations, it appears that the toads will not start breeding below 12° C. but will continue if already in congress when the temperature falls considerably below this. I have had no opportunity to learn the lower limit—that is, how low the temperature must fall to stop a breeding congress already in progress. Since calling males stimulate each other, it seems probable that a small chorus would be stopped by a higher temperature than a large one.

Psychological differences in the sexes during breeding are suggested by my observations, although the facts may be explained as well, perhaps, on the assumption of the differential physiological effects of hormones. Whatever the cause, the result is a well-marked difference in behavior. The following summary of observations will bring out the essentials.

(1) Males always precede females to the breeding sites.

After rains and at the proper temperatures, toads of both sexes emerge from their burrows in large numbers at or just before dusk. The males go immediately to pools of water and start calling. Females usually remain away from the pools for some time. As the evening progresses, more and more males congregate about certain pools and add their voices to the din. In the meantime, some of the females often migrate into adjoining pools, where they may remain for an hour or more, apparently paying no attention to the clamoring males. Later, usually beginning about 9:30 P.M., these females join the males, and mating actually begins (Bragg, 1936a). Some females, however, remain in the grass till about this time and then enter the pools where most males are calling, directly.

(2) Females apparently "prefer" larger pools; males do not. A male of *Bufo cognatus* will often call from a depression which contains a very small quantity of water; I have found them on many occasions calling from pools where, if eggs were laid, there would be little chance for the young to survive. But a male seldom succeeds in attracting a female to such a place. I have seen females actively hopping by calling males and within a few feet of them only to enter a near-by larger pool in which males were calling. Since males commonly call from the edges of pools (Bragg, 1937), occasionally a calling male will intercept a female in or near a small pool. Almost invariably, the female struggles as if in attempt to escape, sometimes succeeding in leaving the pool, taking the male with her. This is the probable explanation for the observation made earlier (Bragg, 1937) that eggs are seldom found in small pools, even after a congress of males has been about them. Sometimes, however, the female loses in the attempt to leave, in which case eggs may be laid. This probably explains, at least in part, why small pools, when they do contain eggs, seldom have more than one clutch each.

It is obvious that these reactions of the females constitute a definite benefit to the race. They insure that more clutches of eggs will be laid where they will have some

chance of developing before the water in the pool disappears through evaporation. In unscientific language, one might say that the female is more responsible than the male. The "problem of the male" is exemplified in the social activities of these toads in much the same sense as in mammals (Wheeler, 1934).

The reaction of the males to the mating calls of one another is also of interest in this connection. The first male to reach a pool starts calling. Other males arriving at this and adjacent pools join in. Inevitably, more males happen to gather about some pools than about others, and usually these are the pools of greatest extent. Within or about any one pool, if one toad starts calling, others immediately take up the chorus, both in this and in near-by pools. The result is that some pools are much noisier than others. As the night wears on, many males about the smaller pools cease calling and join those in the larger pools near-by, so that by 1 or 2 A.M. very few toads remain about the small pools, but the chorus in the larger pools has become greatly augmented. In this manner, large congresses are built up during the course of one night; but on another night, the height of the congress may develop at another favorable place under the same influences. Males are clearly stimulated by each other; and females, other things being equal, are attracted to the region of the loudest clamoring.

While the development of such congresses has been observed many times during the last three years, it was strikingly shown by some observations made during the spring of 1938. Just south of the main campus of the University of Oklahoma there is a polo field which becomes extensively flooded during heavy spring rains. Adjacent to this, on the southeast, is a low field bordered on the east by a deep ditch. On the night of March 28, after a rain of more than four inches, males of *B. cognatus* were very abundant here, calling from the flooded areas of the polo field, particularly in the southeastern portion at 9:15 P.M. At 12:40 A.M. there was less calling and fewer toads here, except at the very southeastern limit of the polo field by

a fence which separates this from the adjoining field. Most of the males had congregated here about a trough-like depression just north of the fence. On the next evening at about 10 o'clock, there were a few calling males in the polo field, particularly about this depression, and many more south of the fence, in the field adjoining. On March 30, from 10 to 11:30 P.M., there were no toads on the polo field, not even about the depression above mentioned, but a very large congress was present south of the fence. On subsequent evenings, the congress of toads moved progressively southward till eventually it was approximately 325 feet from the depression north of the fence where it had originally developed. Breeding was successful in both the depression and in the field farther south, for I collected metamorphosing young in both places late in May.

Amplexation occurs whenever a female comes in contact with a calling male, or with one which has just ceased calling. Males will also clasp other males but release them almost immediately. The reason for this is still unknown (see Bragg, 1937, for notes on this problem). The axillary embrace is used. Egg-laying is a long process, often lasting till well into the day following amplexus. Sometimes, however, it is completed within one night. The eggs are laid, a few at a time, typically in two long strings wound about on the bottom of the pool. The eggs are small (average diameter just less than 1.2 mm). Each is enclosed in a gelatinous capsule, and these capsules of each string of eggs are enclosed by a continuous, tough, elastic, gelatinous tube which serves to protect the eggs. There are about 20,000 eggs in each clutch, but this varies some with the size of the female. For more detail, see Bragg (1937).

At ordinary temperatures, hatching occurs in something over fifty hours (fifty-three in some laboratory cultures maintained in tap-water; Bragg, 1936a). Freezing weather, accompanied by snow, slows development but does not harm the embryos and young larvae in the field. At the opposite extreme, the embryos and larvae develop normally in pools which reach a temperature during the day

of 37° C. This is not necessarily the upper limit of tolerance but only the highest at which observations have been made.

HATCHING

Hatching is a very interesting process. It has been observed in about one hundred embryos, but was studied most carefully upon fifty individuals brought in from the field just as they were about to hatch. The observations were made by means of a binocular microscope.

During embryonic development, the fluid-filled cavity of the egg capsule gradually becomes larger till it reaches 1.76 + to 2.19 + mm in diameter at hatching. For some time the embryo rotates slowly by means of its external cilia, but as the time of hatching approaches this movement becomes progressively slower and finally ceases altogether. The anterior end of the embryo is usually directed upward at this time and the head becomes pressed firmly against the wall of the capsule, which is thus forced against the retaining wall of the outer tube.

The embryo remains in this position for about ten minutes (sometimes less, often slightly more), but its cilia continue to beat. Suddenly, the membranes begin to bulge and a small circular opening suddenly appears in them at the point where the head is in contact with the wall. Immediately the embryo begins to emerge, coming slowly at first and with some apparent effort. The head of the emerging larva becomes distorted by being forced through an opening considerably smaller than itself. The opening gradually increases in size, partially by its edges becoming stretched, but it always remains smaller than the largest diameter of the larva. As the head comes completely free, there is a sudden jerk, caused by the elastic contraction of the edges of the opening as they reach the narrowing portion of the larva's body posterior to the head. The edges of the opening get caught, however, on the bulging posterior portion of the body, and another slow forced movement is necessary to complete the process of hatching. When the edges of the opening reach the narrowing caudal

portion of the animal, they again contract sharply and the larva is projected from the membranes into the surrounding medium.

As noted earlier, the head of the larva is usually directed upward. The animal is, therefore, forced from the membranes against gravity and begins immediately to sink downward. In so doing, it must come close to the membranes from which it has just emerged. In over sixty larvae observed, all but one caught upon the membranes by means of the sticky secretion of the adhesive organ. The larvae are enabled to do this because of the mechanics involved in hatching; the contractility of the edges of the opening in the membranes and the shape of the body of the larva are such that the body is turned at just the right angle as it is projected upward at hatching to fall back with the ventral surface of the head coming in contact with the membranes. Some larvae catch the membrane squarely by their ventral surfaces, but others catch it slightly to one side or the other. In the one exception observed, the larva swung too far to one side, failed to contact the membrane at the proper place, and fell to the bottom of the container.

The whole process of hatching occurs quite rapidly. Only one animal was observed to take longer than one minute from the time that the opening first appeared. The usual time is about one half minute. The exact time consumed appears to depend upon the size of the original opening, although this was not checked carefully.

Absolutely no muscular movements were to be seen in any of the larvae during hatching. I have seen muscular movements both before and just after hatching, so the muscles must be functional at this time. The fact that they are not used during hatching raises the question as to source of the considerable force necessary to push the larva through an opening which is considerably smaller than itself.

My observations show that three factors are involved, two of which are mechanical in the sense that no vital processes are used. These are (1) the elasticity of the

membranes, particularly that of the outer tube; (2) pressure against this elasticity by the accumulation of liquid around the embryo in the capsule; and (3) action of the cilia on the external surface of the body. This last factor is of much less importance than the other two. As evidence, I submit the following:

(1) The space about the embryo increases as development progresses and more fluid accumulates in the cavity of the capsule. (2) the outer tube appears to be very elastic whenever a portion of it is handled. (3) At hatching, there is always a violent contraction of the tube and of the edges of the opening in the tube through which the larva has just emerged, so much so in fact that, if the middle one of three adjacent embryos hatch, one has to look carefully to determine where the middle capsule has been, so closely do the other two embryos approach each other. In some cases one would hardly suspect that a larva had hatched from the appearance afterward. One can, however, often see faintly the wrinkled laminations of the collapsed capsular wall. Also, the whole egg-string for several millimeters on each side is shaken by the emergence of each larva.

The cause of the appearance of the opening through the tube which is utilized at hatching is still not certainly known. All conditions are just as one would expect if a frontal gland were to become active and its secretion were rapidly to dissolve the substance of the wall as described by Noble (1926) for other species.

The percentage of hatching is high under favorable conditions in the field. Of more than fifty clutches observed, only one has been found in which most of the eggs did not hatch, provided the pool was not obviously contaminated. In the one exception, none of the eggs hatched, despite the fact that tadpoles produced earlier in the same pool were developing normally. In the laboratory, frequent changes of water are necessary to avoid injury from products produced by the disintegration of the jellies surrounding the embryos. Crowding in laboratory cultures kills many em-

bryos, perhaps through lack of oxygen, but if the eggs are allowed to remain as left by the parent toads in the pools, they usually thrive.

The variations in developmental rate observed earlier in laboratory-grown embryos (Bragg, 1938) are also evident in embryos growing undisturbed in the pools; but the variations are not so marked and striking. It is fairly certain, therefore, that these embryos, like so many other aquatic animals, can withstand great changes in environmental conditions but are, at the same time, influenced by relatively minute changes. It is also evident that some variation in rate of development is inherent in individual embryos, as maintained earlier.

FACTORS LIMITING NUMBERS

Almost nothing has been published concerning the ecological factors, either physical or biotic, which tend to limit the numbers of individuals of *Bufo cognatus*. As applying to the American toad, Miller (1909) estimated that 85 per cent. of the young are killed as follows:

Non-fertile eggs	15 per cent.
Drying of pools	25 per cent.
Predators	39 per cent.
Fungi	1 per cent.
Other diseases	5 per cent.

Thus, about 15 per cent. of the eggs produced eventually passed through metamorphosis. Of these, 20 per cent. were killed by drying; 25 per cent., by the rigors of winter; 15 per cent., by becoming trapped in sewers and wells; and the remainder by various causes.

General observations indicate that these percentages would be somewhat different for *Bufo cognatus*. Few non-fertile eggs have been found, and my general impression is that 15 per cent. would be too high. The drying of pools, however, is a most important factor in the death of eggs and larvae. For example, during 1936 and 1937, no toads of this species succeeded, in the vicinity of Norman, in effective breeding because of this factor alone, although

at least some eggs were laid during the spring of each of these years. In 1938, tadpoles in smaller pools met a similar fate, but those in the larger pools had ample time to pass metamorphosis. I have no clear idea as to what percentage of young so destroyed would be over a long term of years, but I am certain that it would greatly exceed 25 per cent. This is, of course, what could be expected in the drier habitat of central Oklahoma, as compared with central Massachusetts, where Miller's work was done. The comparative scarcity of these toads in still drier regions (*e.g.*, western Oklahoma, Arizona) may well be accounted for in large part by the failure of breeding during most seasons to be effective because of this one factor.

Predators play an important role in reducing the number of tadpoles. The spadefoot toad, *Scaphiopus hammondi*, often breeds in buffalo wallows. Its tadpoles are much larger, more active and develop at a much faster rate than those of *Bufo cognatus*; and they are carnivorous. Trowbridge and Trowbridge (1937) presented indirect evidence that these large tadpoles feed upon the smaller *Bufo* larvae, but they had never seen a direct attack. On several occasions, I have seen a *Scaphiopus* larva attack, kill and devour a tadpole of *B. cognatus*, and in one instance believe them chiefly responsible for the complete disappearance of *Bufo* larvae from a pool. Predaceous beetles also take a heavy toll. *Hydrophilus triangularis* invaded the buffalo wallows in large numbers in 1938, and its larvae were seen feeding upon tadpoles. At present, these are the only known predators, but I have reason to suspect other animals. I have several times found the terrapin (*Terrepenne ornata*) in and about the breeding pools, but never was able to prove that it ate either eggs or larvae. The nature of the breeding sites precludes the possibility of fishes, most other amphibians, or aquatic reptiles as predaceous enemies.

During metamorphosis, the tiny toads are quite defenseless, and one would expect them to fall prey to many animals, especially to birds. Dr. Charles Smith informs me

that he has seen crows picking up young toads from the edges of buffalo wallows on two occasions. Since no other toad in central Oklahoma uses buffalo wallows extensively for breeding, it is very probable that these were *B. cognatus*.

I have never seen evidence of fungous or other diseases attacking eggs or larvae; but fecal contamination of the water in overgrazed pastures sometimes kills all the eggs produced in a given pool (Bragg, 1937).

Little is known about the enemies of adults. Dr. A. I. Ortenburger informs me that the hog-nosed snake (*Heterodon*) is very fond of toads. Since snakes of this genus are common in the habitat of *B. cognatus*, it is probable that some individuals fall prey to them. Black snakes and bull snakes eat *B. w. woodhousii* and, very likely, *B. cognatus* also. Dr. Charles Smith recently shot a large hawk which had just caught a toad that it was eating. The victim, a female of *B. w. woodhousii*, was still alive. It seems likely that hawks might also catch *B. cognatus*, particularly those active in the daytime.

The automobile kills hundreds of toads each year. I have seen as many as fifty dead toads along a mile of paved road after a rain. Many are killed also in the unpaved country roads about Norman, especially during the breeding season.

The Great Plains toad, like its eastern relative, often becomes trapped in pits from which it can not escape. In one case, more than twenty-five were taken from a well-like depression. I have also found them trapped in post-holes. It is doubtful if they often fall into sewers, as does the American toad, because they frequent human settlements very little.

Apparently, very few of these toads are killed by winter. At least, thousands were present during breeding in the spring of 1938 in a region where they had not bred successfully for at least two seasons. If winter-killing were common, one would expect the numbers of toads to be visibly depleted after three winters had passed without breeding.

The parasites of *B. cognatus* are very imperfectly known. Trowbridge and Hefley (1934) found a single individual to harbor a heavy infestation of intestinal Protozoa (*Opalina* sp.). A cestode, referred to *Ophiotaenia magna*, was also found. No trematodes, nematodes or arthropods were seen. Dr. J. Teague Self has noted heavy infestations of intestinal Protozoa in several individuals. He has also found a cestode (*Nematotaenia americana* Jewell, *Distoichometra bufonis* Dickey, or a form closely related to one of these) which occurs commonly in the small intestine. More knowledge is badly needed upon the distribution, life-histories and the effects of parasites of toads.

(To be concluded in the September-October, 1940 issue)

RADIATION AND THE HEREDITARY MECHANISM¹

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IN this symposium on "Radiation and Life," I have been assigned to talk about the influence of radiation on the hereditary mechanism. By this we mean effects on genes, chromosomes and on the chromosomal mechanisms accompanying meiotic and mitotic cell division, which form the visible basis of the phenomena studied in genetics. It has been found that these structures are affected by all those kinds of radiations that produce ionizations, such as x-rays, radiations from radioactive sources and neutrons, which do not themselves produce ionizations, but which do so by virtue of the recoil protons arising in any hydrogenous material. Within wide limits it has been found that the type and the rate at which these effects are produced are independent of the specific kind of radiation and are only a function of the number of ions which these radiations produce in a given volume, so that for most purposes only the total dose of ions need be considered. In addition, ultraviolet light below 3,000 AU has been found effective, although, as we are now beginning to learn, its effects may differ qualitatively in some important respects from those produced by the ionizing radiations. The ultraviolet light in such experiments attacks proteins in their aromatic amino acids and nucleic acids in their pyridine groups. The ionizing radiations attack any molecule without preference. It is therefore plausible that the ionizing radiations should be able to cause a wider variety of types of effect.

Nevertheless, both the ionizing and the ultraviolet radiations should be grouped together in two important re-

¹ Invited address as part of a symposium on "Radiation and Life" at the meeting, at Stanford University, of the Pacific Division of the A. A. A. S.

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spects. First, their primary action is unphysiological. Their primary action consists in either destroying or changing the function of the molecule which they attack, in striking contrast to the action of light in photosynthesis or in visual perception. Second, they both can and generally do hit by an all or none law, in which a single ionization or absorption produces the entire macroscopic effect independent of all other ionizations and absorptions. This feature stands in contrast to normal physiology where we invariably deal with large numbers of molecules of each kind, and where the elimination of a single molecule could therefore never result in observable effects. It is, however, intimately related to the unique position of the genes in cell physiology, for which it appears to be characteristic that each type is only present in one specimen per chromonema.

In order to appreciate the significance of this last point clearly, let us consider a crude model: A cell may contain a thousand molecules of a certain enzyme which may be connected with its respiration or other metabolic functions, and let us assume that one half of these molecules, 500, would have to be knocked out in order to render the cell non-functional. Let us assume further, that the cell contains 10 "genes," that is, molecules, perhaps in size similar to the enzyme molecules, but only one specimen of each kind, each having a different function in the cell, so that a change in any one of these genes will change the entire cell; for simplicity of discussion we will say that all changes are lethal. Let us now irradiate this cell with an "unphysiological" radiation. Let us choose a dose which knocks out one fifth of the molecules in question, *i.e.*, there is a chance of one in five for each molecule to be knocked out. The effect on the enzyme system will be negligible, only 20 per cent. being eliminated. That may show up for a while in the metabolic activities, but it is not a fatal injury. Therefore, no cells will be killed by the action of this dose on the enzyme system. On the other hand, the chance that in a given cell no gene will be hit, is

$$(1 - \frac{1}{5})^{10} = .13$$

That is, only 13 per cent. of the cells will have survived this dose because of the action of the dose on the genes. The survivors will have their genes quite unimpaired. Note that this is not caused by a greater sensitivity of the genes as such, but merely by their unique standing in the cell organization. Let us see how the survival fraction will depend on the dose. For each individual molecule the probability of not being hit will decrease exponentially with increasing the dose. This is a simple consequence of the quantized nature of the elementary processes involved. For the cell as a whole we will see, therefore, that the survival fraction decreases exponentially, only with a ten times greater decay-constant, corresponding to the ten times greater sensitive volume of the cell as compared with that of the individual gene. For sufficiently small doses, the number of cells killed by the action on any of the genes will be proportional to the dose. There will be no threshold value; even the smallest doses will kill a correspondingly small fraction of the cells. Only for very large doses, when a considerable fraction of all molecules are harmed, will there be interference with the normal physiological reactions involving large numbers of molecules. Then the exponential law of decrease of survivors will be replaced by a threshold effect, the remaining cells being finished off uniformly.

This peculiar type of dose-dependence of the harmful effects, an all-or-none law involving increasing fractions of the cells, brings the radiation effects on the hereditary mechanism in a class with still other types of phenomena: the inactivation of enzymes by radiations, of viruses, of bacteriophages, and, most important, the killing of bacteria. This was shown by experiments of R. Wyckoff (1930; 1932), of A. Hollaender (1936) and of Haines, Coulson and Lea (1937), for all types of ionizing radiations and for ultraviolet light. Hollaender and Emmons (1939) have even succeeded in producing mutant forms of a spore-forming non-sexual fungus. For enzymes, viruses and bacteriophages all this is readily understandable;

these represent single molecules themselves, while the results for bacteria prove that these cells also contain gene-like centers in the sense that they can not function if certain individual molecules are rendered non-functional. It appears that these conclusions for bacteria are borne out by a still wider class of experiments, namely, the killing of bacteria by disinfectants or by heat. Also by these agents, the first effects appearing upon small "doses," is a killing of a dose-proportional fraction of individuals and with no effect on the rest. This again must be interpreted as interference with controlling individual molecules governed by statistical laws. These implications of a large body of hitherto somewhat uncorrelated observations have recently been emphasized by P. Jordan (1939) in several important publications.

Now let us begin at the beginning with the simplest case, the inactivation of an enzyme by ultraviolet light. The best work on this problem was done by Kubowitz and Haas (1933), who investigated the hydrolytic enzyme urease, the molecular weight of which lies around 400,000. They found that this enzyme was inactivated in a wide range of ultraviolet, from 1,960 AU to 3,660 AU. In this range the absorption coefficient changes by a factor 5,000, the inactivating rate being nearly proportional to the absorption coefficient. The quantum yield of inactivation, however, is not equal to one but is quite small, about one in 250, *i.e.*, on the average, only one out of 250 absorbed quanta inactivate a molecule. It appears that the absorption of the protein in this range is predominantly due to the absorption of the aromatic amino-acids. The primary effect may consist in the splitting of the adjacent peptide bond, since we know from a model experiment of D. C. Carpenter (1939) that this can occur. These results should be interpreted to mean that of the many amino acids present, only one or a few are situated so that an absorption by them entails inactivation of the enzymic capacity. In the region around 2,600 AU, where most of the other UV-work has been done, this requires a dose of about $2 \cdot 10^6$ erg/cm² for the destroying of half the activity.

In the case of pepsin, the measurements of Gates (1934) indicate that a bigger dose of $5 \cdot 10^7$ erg/cm² is required for half inactivation. Pepsin is a very much smaller molecule than urease, and it may be that its greater resistance is connected with its smaller size.

In the case of a virus we have the measurements by Hollaender and Duggar (1936) on the inactivation of the tobacco-mosaic virus. Here the dose for half inactivation is only $1.6 \cdot 10^9$ erg/cm². In comparing this with the inactivation of enzymes we must, however, bear in mind that the effect measured by the experiment is a different one, namely, the loss of the capacity to produce lesions in the host and that probably means the loss of the capacity to reproduce. It is indeed very plausible that this capacity for reproduction should be more vulnerable than the enzymatic faculty. The latter depends on the activity of selected areas in the molecule, and a change in a distant part may not interfere with it. Whereas in reproduction, the entire molecule is necessarily involved and its vulnerability should then increase in proportion to its size. I have found similar values for a bacteriophage, which is in keeping with the close relationship between these two types of particles.

With bacteria and ultraviolet light several authors have done valuable quantitative work—Wyckoff (1930; 1932), Hollaender and Claus (1936) and Herčík (1937). They all find the exponential decrease of the survival fraction, and a dose of about $2 \cdot 10^4$ erg/cm² to kill half of the irradiated sample. This is again a considerably smaller dose than that required for viruses but is not, by far, in proportion to the very much greater size of these organisms. It can be explained by assuming that these cells contain a number of controlling particles of virus-size. Hollaender and Duggar (1938) in a most interesting series of experiments have also been able to detect and to analyze some apparently purely physiological effects of ultraviolet light on bacteria. The main effect is an extension of the normal lag-period. It is measurable only with doses that leave but a small fraction of the organisms alive. Very prob-

ably we have here before us the direct effects on some enzyme system, involving large numbers of molecules, so that the effect is dose proportional in each cell.

Up until now we have confined our attention to an analysis of effects of ultraviolet light. The effects consisted almost exclusively in destruction, inactivation of enzymes, of viruses, and killing of bacteria. In view of what we shall see later about the effects of radiation on genes I think it is probable that the limitation to these extreme effects is solely due to the technique of detection. In reality, there should also here be many cases of "viable mutations." The experiments mentioned so far afford proof of the importance of individual atomic groupings for the whole organization and the comparison of the absorption spectrum with the inactivation-spectrum can sometimes give a hint as to the nature of the primary photochemical process. An essential prerequisite to this success was the fact that these objects are so small that they absorb only a little of the impinging radiation. The intensity of radiation at the sensitive points is therefore equal to the outside intensity and can be evaluated with accuracy, and the relation between dose and effect can be worked out quantitatively. For these reasons ultraviolet work is more rewarding than the work with ionizing radiations on these objects and much can be hoped from further refinements in method and from collaboration with photochemistry.

When we turn to higher organisms, the situation changes with respect to all these last-mentioned points. We lose the possibility of measuring the intensity of the ultraviolet light at the sensitive points because it is not sufficiently penetrating. In most cases, it is not even possible to get any effective doses to the critical points. For these reasons the ionizing radiations have been more important for the study of effects on the hereditary mechanisms. For these radiations there is no analogue to the spectral analysis which is so helpful in the case of ultraviolet light, but this lack of finesse on the part of the physical agent is amply made up for by the infinitely greater

variety of phenomena that are produced on the biological side.

The macroscopical effects that the experimenter can observe are all concerned with the nucleus of the cell. They can be seen either by direct inspection of the irregularities produced in the chromosomal mechanism of the irradiated cell or of its offspring, or, indirectly, by a genetic analysis of the F_1 or F_2 of irradiated parent-individuals or parent-gametes. It is very important to bear in mind that even the apparently most direct effects of radiation, those observable in the irradiated cell itself, are a long way removed from the initiating ionizations. Indeed, the atomic physicist would class anything the cytologist can see with his instrument as macroscopic.

The first thing we have to decide for any given effect is its dependence on the dose. Is it an all or none effect, where the fraction of affected cells depends on the dose (in simple cases being directly proportional to it), or is it a graded effect, more or less uniform on all cells, where the grade depends on the dose. In the first case, we speak of a single hit effect (the hit need not be a direct one, *i.e.*, the ionization need not take place at the affected gene); in the second case, we speak of a mass effect. For bacteria we saw that the single-hit effects were easily measurable at doses at which the mass-effects were still small. In higher cells this is not necessarily so. If growing tissue is irradiated, it appears that nuclear divisions that were just about due to start are delayed by a time that is perhaps proportional to the dose (Carlson, 1938). This would seem to be a typical mass-effect. We might here assume that part of the substances controlling the division are destroyed, so that the rate of the reactions in which these substances take part is decreased. However, the very low dose (100 r and less) at which this effect is quite pronounced suggests a somewhat different interpretation, namely, that there are a very large number of parallel reactions each of which can be delayed, possibly each by a single hit, and that the observable delay in mitosis is a summative effect in the sense that each, sev-

erally, of the inhibitions have to be removed before mitosis can proceed. In this case we should expect that the delay is proportional to the logarithm of the dose, rather than to the dose itself. The experimental data are not yet sufficient to decide this point. This picture would link the effect to the typical single-hit cases.

As soon as the new mitoses appear, a variety of aberrations can be seen: broken chromosomes, broken chromatids, fusions of broken pieces, reciprocal translocations and still more complicated rearrangements in which more primary breaks are involved. The comprehensive and elaborate analysis by Bauer, Demerec and Kaufmann (1938) of giant chromosome rearrangements, of Sax (1938) on *Tradescantia* microspores, of Carlson (1938) on mitoses in the neuroblasts of a grasshopper, and of Muller, Makki and Sidky (1939) on dose-dependence of complex rearrangements in giant chromosomes of *Drosophila* have contributed much to a clarification of the sequence of events once the effect has increased from the atomic to the chromosomal order of size. About this intermediate phase we are still entirely dependent on conjectures.

The aberrations start as simple breaks of chromosomes or chromatids; the broken ends then have a strong tendency to recombine with other broken ends but not with free, unbroken chromosome ends. This recombination is limited in space and time so that in cases where many primary breaks are produced recombinations tend to occur in pairs and not entirely at random. However, at least in *Drosophila*, the spatial arrangement of the chromosomes in the sperm nucleus appears to vary sufficiently from cell to cell so that there is no preponderance of recombination of any specified region with any other.

The appearance or non-appearance of identical breaks in two sister chromatids has been taken as indicating whether the chromosome in question had already split at the time of irradiation. The argument was twofold: (a) since the break is caused by a single ion pair which can not act at two morphologically different points, sister-breaks indicate doubleness at time of irradiation, and

conversely; (b) in cases where only one of two sister chromatids is broken the lesion could not have applied to the original single chromosome. The application, however, of these criteria has led to difficulties. Sax (1938) found that sister-breaks and single breaks can occur in the same cell, even in the same chromosome. Although it is true that irradiation at early stages of the cycle causes more sister-breaks and vice-versa, the two phenomena overlap over a long range, seemingly requiring a lack of coordination between different chromosome-regions in performing the duplication. Sax has therefore advocated the view that the first of the above arguments was invalid; sister-chromatids should both be breakable by the same single hit. I do not think that such a view is tenable on physical grounds. One might try to sacrifice the second argument (that a single chromatid-break proves doubleness at the time of irradiation). It seems natural to assume that if a chromosome is broken and doubles afterwards, one of the chromatids may reunite thus giving the impression of a single chromatid-break. In this case, however, we should expect a minimum fraction of single chromatid-breaks however early we irradiate, and this is not borne out by Sax's observations. At present, therefore, I see no alternative but to interpret Sax's findings on their face value—saying that the duplication really does proceed with little coordination. We would then have to assume that the comparative uniformity in the timing of the cycle in different cells was again the outcome of its dependence on many individual reactions.

With respect to deficiencies, Demerec, Kaufmann and Hoover (1937-38) have found a very important result. Making a statistical analysis of the lengths of deficiencies which are produced by x-rays, they find a bi-modal distribution indicating two different types of deficiencies. The long deficiencies, which are uniformly distributed over all lengths and should be attributed to two independent breaks, with elimination of the intercalary part—and the short deficiencies, involving only one or a very few bands and which on statistical grounds must be ascribed to single

hits. This latter class is interesting theoretically, since it seems to show how very far the effects of a single hit can spread on the chromosome. We should, however, bear in mind that this spreading is not an immediate effect. It may well be connected with the difficulties of coordination which must confront a chromosome, one gene of which has been rendered incapable of reproduction.

Demerec and Hoover (1937-38) have also recently contributed a very valuable cytological study of a large number of genetical changes caused by x-rays in a picked region of the X-chromosome of *Drosophila melanogaster*. They found all viable changes to be free of chromosomal aberrations and deficiencies. Also of the recessive lethals the majority showed no chromosomal rearrangements, although many of them showed some deficiencies—the majority belonging to the one-hit class. This result explains why it was possible to find the linear relationship between dose and mutation rate for these classes of mutations, since we see now that they are of a type which stands apart from the more complex effects which eliminate a considerable fraction of all irradiated cells before they can be analyzed genetically.

This brings us at last to the mutations proper. As you all know, it was the startling announcement by Muller in 1927 that large numbers of these could be produced by x-rays in *Drosophila*, and the independent work of Stadler with similar results in barley, which started off the vast amount of research of which I could only give you such a very deficient account. The first thing that could be established about the effect was its proportionality to the irradiating dose, and this, as we have seen, has survived the sometimes obscuring complexities of later and more elaborate research. The proportionality rule gave the basis for the single-hit interpretation and for the calculation of sensitive volumes (Timoféeff-Ressovsky, Zimmer and Delbrück, 1935). These sensitive volumes were found to be quite large on the atomic scale—comprising several hundred atoms even in cases where a well-defined mutational step is considered—like that which changes

the eye-color from normal to eosin, an intermediate shade. Recent experiments with neutrons by Timoféeff-Ressovsky and Zimmer (1938) support the view that these sensitive volumes represent well-defined areas and that the effect is really a direct one on the gene. Such large sensitive volumes could easily be explained by analogy with inactivation of enzymes which, as we saw, react similarly. We would say that the primary chemical change was rather unspecific in causing a given phenotype response, many "chemical mutations" showing the same phenotype. That would leave out of consideration, however, the important class of back-mutations, for which we have no analogue from enzyme-chemistry. They should be very much rarer than they have actually been found to be. Possibly a careful cytological investigation of such reverse mutations could throw more light on the problem.

For some time the relation between point-mutations and chromosome-mutations has been under discussion. The cytological analysis showed an apparently uninterrupted series from the gross rearrangements to the minute deficiencies which in the genetic analysis are not any more distinguishable from point-mutations. We now know at least that we have to distinguish between the two-hit and the one-hit cases. We also know that the one-hit cases may show visible, *i.e.*, macroscopic, changes in the chromosome. Another important distinction appears to emerge from the work of Stadler (1936) on the genetic effects of ultraviolet light in maize. His results show that ultraviolet light does not produce translocations. From this we conclude that it does not break the chromosomes. It does, however, produce deficiencies and mutations. For these the primary chemical change must therefore be different from those which produce breaks. These findings are corroborated by more recent work of Muller and Mackenzie on *Drosophila* (1939). But the question remains, whether all the one-hit mutations and deletions are of this macroscopic type or whether there is another subdivision between this type and such cases, where the

mutation proper is confined to a simple chemical change affecting only one radical, and which is propagated as such. At present we can not even give a tentative answer to this question because we are still so ignorant concerning the primary activities of the gene. We do not know what particular structural qualifications enable a gene to multiply on the one hand and to affect the phenotype on the other hand, if, indeed, these two functions can be separated. We know that for viruses and bacteriophages these qualifications must be quite exacting since their capacity to reproduce is so easily destroyed by radiation. But it is perhaps not permissible to infer that the same holds true for genes, since the reproduction of the gene is a normal function of the cell, whereas viruses and bacteriophages may have to enforce their reproduction by preliminary reactions, and it may be that it is these which are inhibited by the radiation. For instance, in the case of the bacteriophage we know that a prerequisite to its reproduction is its specific absorption into the host-bacterium. We will first have to find out whether or not the radiation is primarily interfering with this capacity to be adsorbed.

In giving you this brief survey of modern radiation work in biology, it has been my intention to emphasize the close interrelationship of the observed effects in a wide range of biological material. They put into striking relief the very peculiar organization of the cell in which the contribution of individual molecules plays an essential rôle. We see emerging here a new field of physiology, beyond that of enzyme-chemistry, which at present enjoys such splendid success in the hands of organic chemistry. In this new field a new feature, that of statistical fluctuations always attendant on small numbers of independent entities, must become of paramount importance. At present we do not know how the cell manages to exhibit such outward regularity in spite of the molecular incoherence. This problem will doubtless come into great prominence as we learn more about its details, and may require new conceptual ways of approach. But even as we enter this new territory, we are rewarded at every step with new insights into

the wonderful mechanics of the hereditary mechanism, for the exploration of which radiation has furnished a powerful tool.

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LONGEVITY IN *DROSOPHILA MELANOGASTER* AND ITS EBONY MUTANT IN THE ABSENCE OF FOOD

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INTRODUCTION

THE effect of the absence of food on longevity has received much attention in recent years. Rau (1910) made observations concerning the duration of life in the saturniid moth, *Samia cecropia*, an insect that does not take food during its imaginal stage. Working with small samples, he reported that the mean length of life of the male was approximately 11 days and that of the female approximately 8 days. Rau and Rau (1912, 1914) reported that the mean length of life in various genera of the saturniid moth ranged from 6.53 days to 15.74 days. They found that the males of *Samia cecropia* (obtained from New York) lived longer than the females, that the males and females of *Tropaea luna* and *Samia cecropia* (obtained from St. Louis) showed no significant differences in mean duration of life, while the females of *Callosamia promethea* and *Telea polyphemus* lived longer than the males. Baumberger (1914) reported studies on longevity in insects without food. Many genera and species were used in this work. The imagoes were caught in a net and for this reason the true dates of hatching and consequently the true ages of the organisms at death were not known. Baumberger, however, concluded from his data that duration of life varies inversely with temperature and is not correlated with systematic groups. Kopec (1924) worked with *Lymantria dispar*, an insect which does not take food during the imaginal stage. He reported that intermittent starvation of the larvae produced a prolongation of the larval period but did not affect the mean duration of life of the adult. Pearl and Parker (1924) reported exact quantitative studies on the duration of life under condi-

tions of complete starvation of two varieties of *Drosophila melanogaster*. They found that under starvation the mean duration of life was almost the same in the vestigial as in the wild-type fly, although in a previous paper (Pearl and Parker, 1921) it was found that under conditions of full feeding the wild-type fly lived approximately three times as long as the vestigial. It was found that density of population had little effect on the length of life of the combined sexes under conditions of starvation. This finding is also in contrast to the results obtained when the flies were fed (Pearl and Parker, 1922). At all densities tested the females had a greater mean duration of life than the males. Lilliland (1938), working with *Drosophila pseudoobscura*, varied temperature, humidity and density of population. It was reported that without food the mean duration of life was greater at lower temperatures, lower densities and greater humidity. It was also reported that Race A lived longer than Race B. The differences were more pronounced at higher humidities.

Lutz (1915) used *Drosophila ampelophila* (*melanogaster*) in experiments dealing with duration of life. He allowed the flies to gain access to water but not to food. The results obtained when compared with more recent work (Pearl and Parker, 1924) showed that the addition of water increased the mean length of life. Loeb and Northrop (1916) performed the same experiment and reported that the mean duration of life varied inversely as the temperature between 9° C. and 34° C.

The reader is referred to Pearl (1928), Alpatov (1930), Pearl and Miner (1935) and Cowdry (1939) for more extensive bibliographies dealing with duration of life.

In the following investigation the duration of life of the wild-type fly of *Drosophila melanogaster* and its ebony mutant in the absence of food and water was studied. The wild-type fly was used as a control. The ebony mutant was used because, as was pointed out to the author by Dr. E. S. McDonough, its cultures thrived hardily under regular laboratory conditions.

The writer wishes to express his sincere appreciation to

Dr. E. S. McDonough, of the Department of Biology of Marquette University, for his constant help and encouragement throughout the course of this investigation. He wishes also to thank Dr. Raymond Pearl, of the Johns Hopkins University, for his helpful criticisms.

MATERIALS AND METHODS

Experiments dealing with duration of life require that environmental factors be constant. A modified two-shelf incubator much like that of Bridges (1932) was constructed to maintain constant temperature. The thermostat and relay assembly was constructed according to Greiff (1939). The incubator during the course of the investigation ran constantly for five months with a temperature fluctuation of $\pm 0.05^{\circ}\text{C}$.

Pearl and Parker (1924) have pointed out the importance of controlling humidity so that there is no water present and at the same time no active desiccation of the flies. Accurate humidity control was accomplished by employing a saturated salt solution, as described by Obermiller (1924). Ammonium chloride (C.P.) was used. This solution has been reported to maintain a humidity of 79.3 per cent. at 25°C . (International Critical Tables, 1926). The solution was poured into a container and a mark made on the side of the container indicating the level of the solution. Care was taken to add only enough water to bring the solution level up to this mark. Crystals of ammonium chloride were added in excess to provide a margin of safety.

The stock cultures from which the flies used in this investigation were obtained were brought to Marquette University from Michigan State College in 1929. The flies were inbred for this investigation and the F_4 generation used. They were grown on a banana-agar medium and kept in the incubator at all times except as noted below.

The pupae comprising the F_4 generation were removed from the culture bottles by means of a flamed nichrome wire. The pupae were washed in 70 per cent. alcohol and

then put into individual test-tubes which had been previously plugged with cotton and sterilized. Each tube contained a strip of slightly moistened paper toweling upon which the pupa was placed. The tubes were placed in numbered racks and examined every 12 hours.

The technique used in handling the flies while they were in the incubator was that developed by Powsner (1935). Only four racks, each containing 30 test-tubes, were removed from the incubator at one time. It was found that no individual rack was out of the incubator more than 15 minutes in 24 hours.

DATA

Tables I and II give the survivorship distributions of

TABLE I

SURVIVORSHIP DISTRIBUTIONS OF *DROSOPHILA* IN THE COMPLETE ABSENCE OF FOOD BASED ON 1,000 FLIES. SEXES COMBINED

Age (in hours)	Wild type	Ebony
6	1,000	1,000
12	998	1,000
24	997	998
36	956	997
48	562	932
60	121	899
72	14	319
84	4	78
96	0	13
108		2
120		1
132		0
Absolute no. of flies	766	784

TABLE II

SURVIVORSHIP DISTRIBUTIONS OF *DROSOPHILA* IN THE COMPLETE ABSENCE OF FOOD BASED ON 1,000 FLIES. SEXES SEPARATE

Age (in hours)	Wild type		Ebony	
	Male	Female	Male	Female
6	1,000	1,000	1,000	1,000
12	997	1,000	1,000	1,000
24	997	998	980	1,000
36	973	940	980	997
48	646	487	930	936
60	151	98	723	702
72	16	17	313	326
84	0	7	77	79
96		0	14	11
108			2	3
120			0	3
132				0
Absolute no. of flies	370	396	441	343

the wild-type fly and its ebony mutant. The tables were

calculated on the basis of 1,000 flies and were corrected to the nearest whole number. The survivorship lines are compared graphically in Figs. 1 to 3. Figs. 4 and 5 show

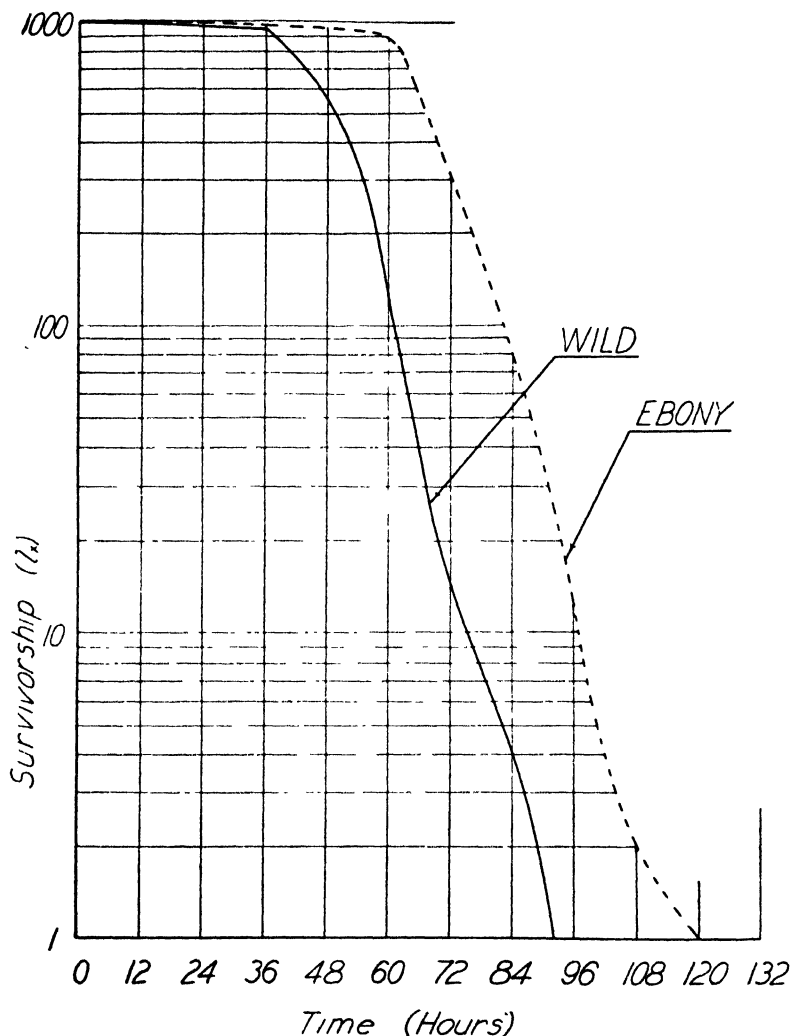


FIG. 1. A Comparison of the Wild Type and Ebony Mutant l_x Lines of *Drosophila*.

the death curves (d_x) and the survivorship curves (l_x) placed on a relative time base for the purpose of comparison (Pearl, 1927).

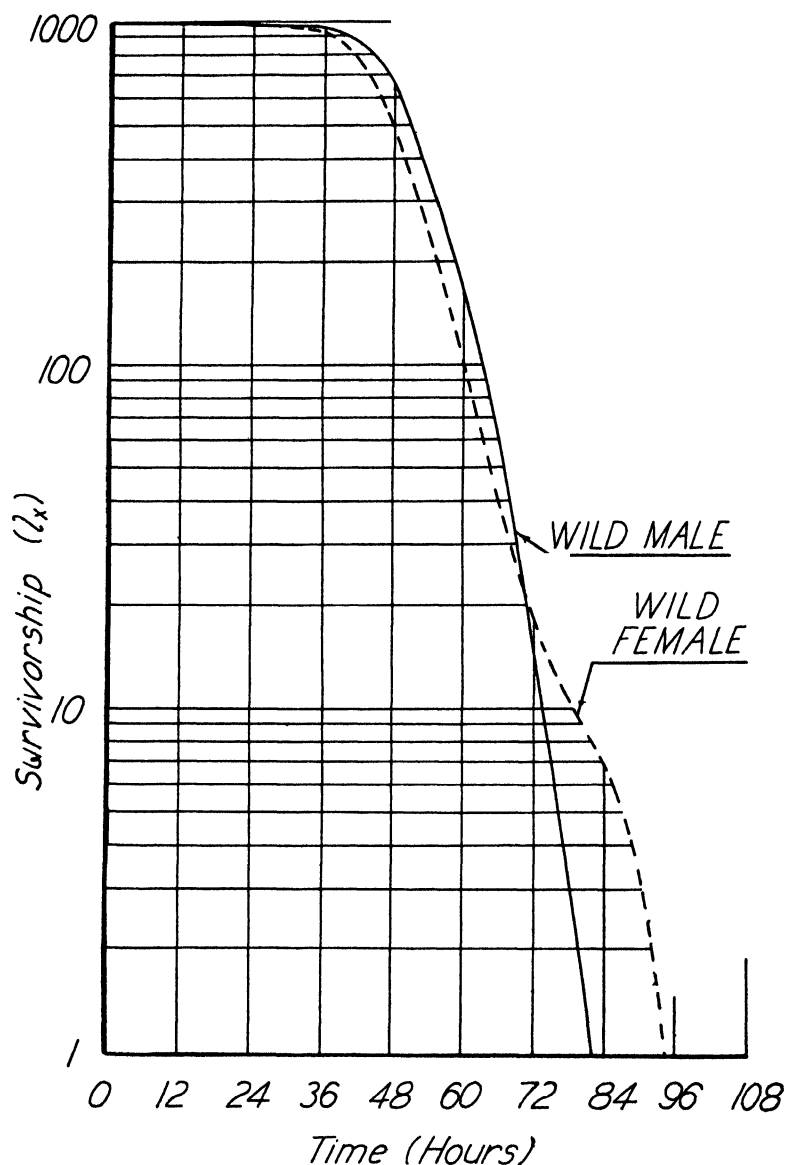


FIG. 2. A Comparison of the Male and Female l_x Lines of the Wild-type *Drosophila*.

The chief biometric constants for this investigation are given in Table III.

The mean duration of life of the ebony fly, sexes com-

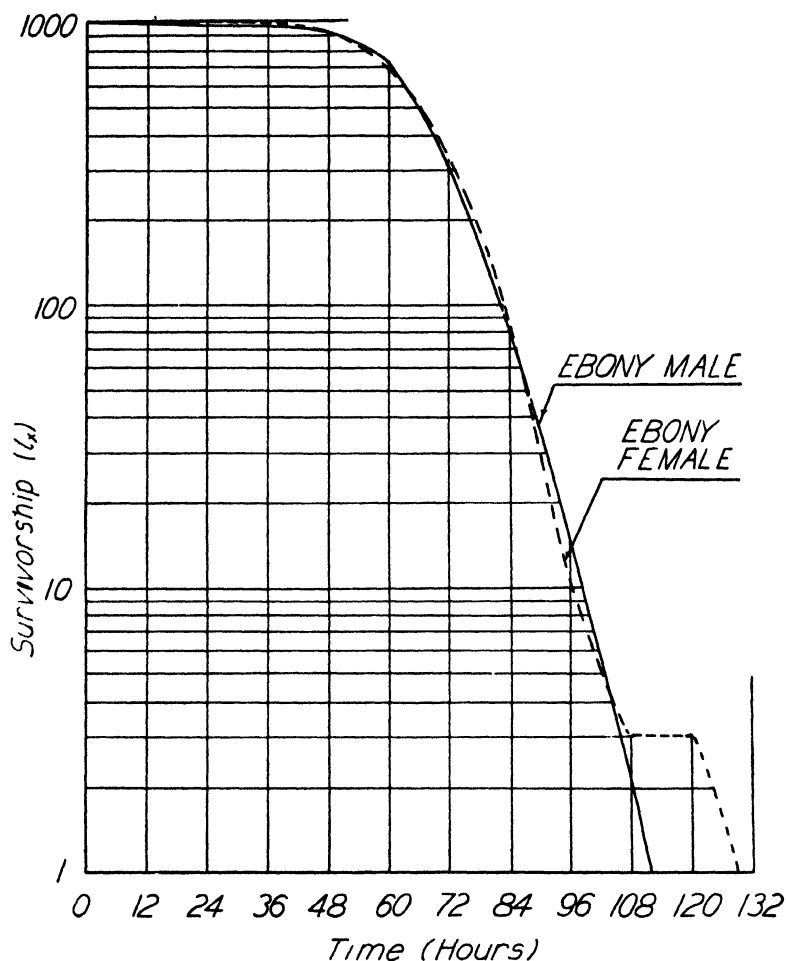


FIG. 3. A Comparison of the Male and Female 1_x Lines of the Ebony Mutant of *Drosophila*.

TABLE III

	Mean duration of life (hours)	Standard devi- ation (hours)	Standard devi- ation of mean (hours)
Wild-type fly (sexes combined) .	49.89	9.77	.353
Ebony fly (sexes combined) . . .	66.76	11.77	.420
Wild-type male	51.51	9.46	.492
Wild-type female	48.91	10.08	.507
Ebony male	66.80	12.82	.609
Ebony female	66.89	12.99	.702

bined, was found to exceed that of the wild-type fly, sexes combined, by 16.77 hours. The standard error of the

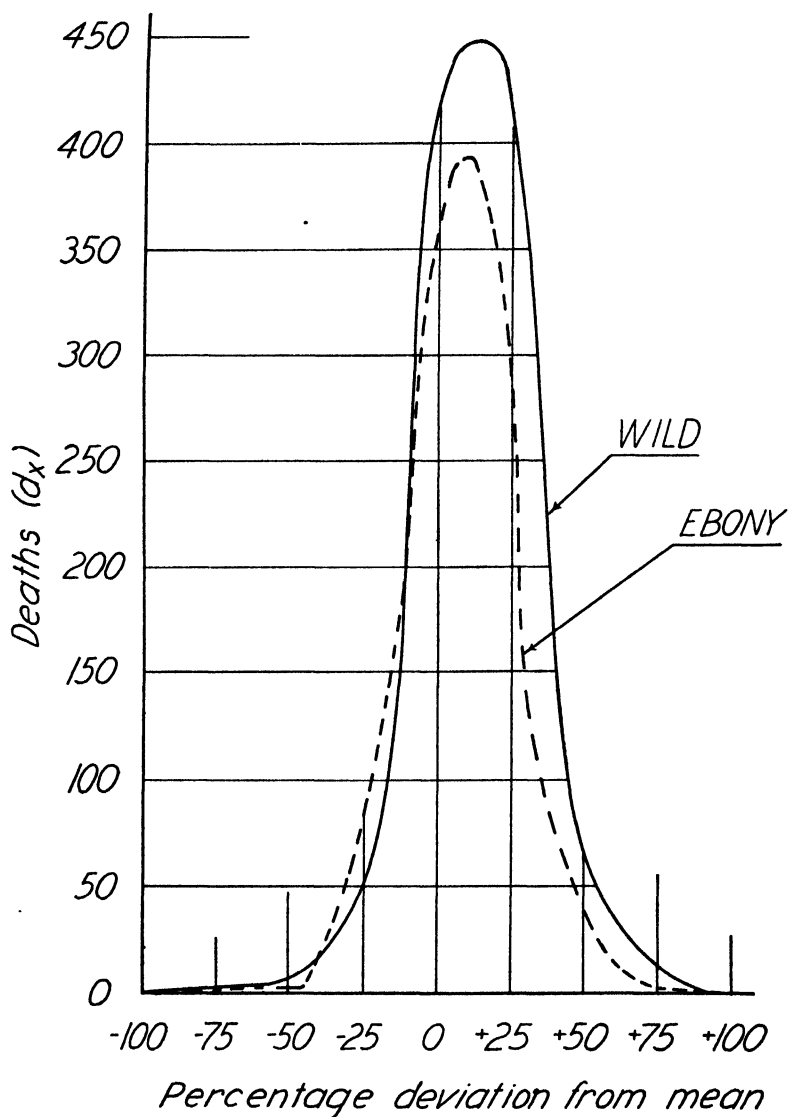


FIG. 4. Death Curves for Wild Type, Sexes Combined, and Ebony Mutant, Sexes Combined, on a Relative Time Base.

For each form represented the mean duration of life is taken as 100 per cent. on the abscissal scale, and all other ages (time duration) are expressed as percentage deviations (plus or minus) from this mean.

difference being .55 the difference was significant. In the foregoing and the following determinations of significance

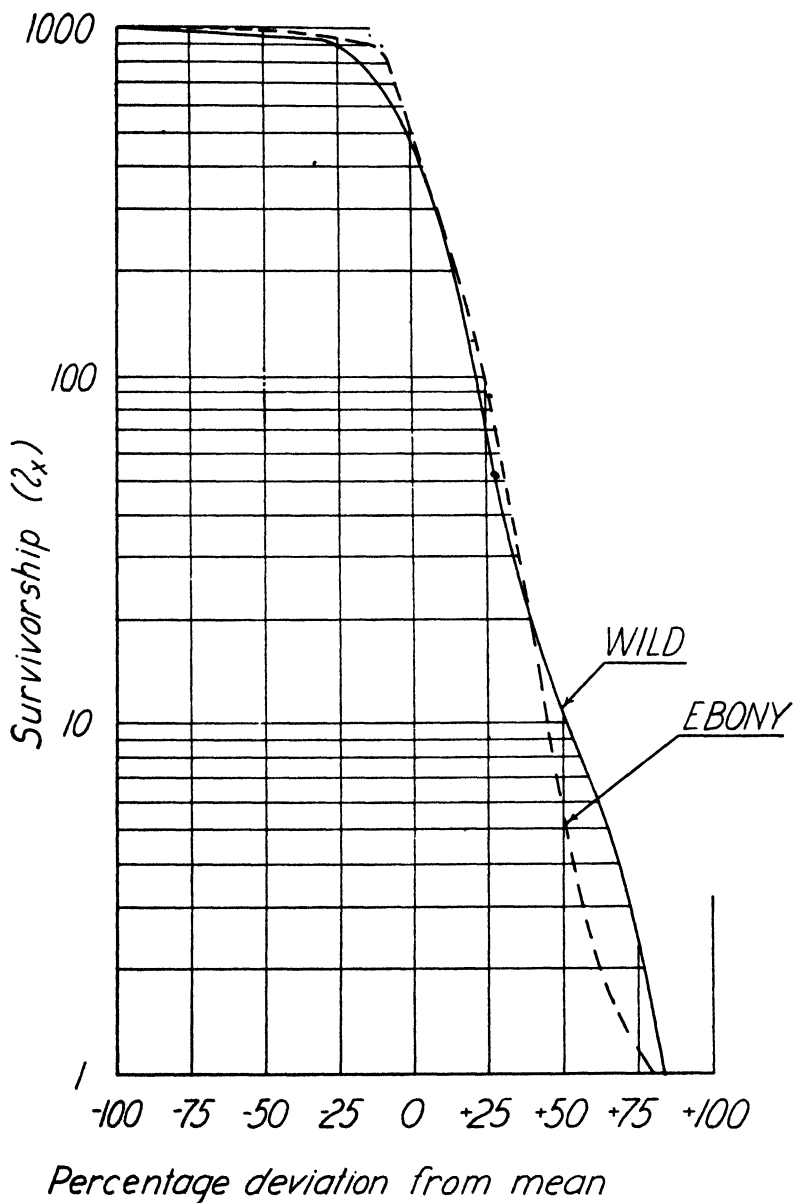


FIG. 5. Survivorship Curves for Wild Type, Sexes Combined, and Ebony Mutant, Sexes Combined, on a Relative Time Base.

For each form represented the mean duration of life is taken as 100 per cent. on the abscissal scale, and all other ages (time duration) are expressed as percentage deviations (plus or minus) from this mean.

or non-significance "Student's" *t*-test for unique examples was used.

The mean duration of life of the ebony female was found to exceed that of the ebony male by .09 hours. The standard error of the difference being .92 hours, the difference was not significant.

The mean duration of life of the wild-type male was found to exceed that of the wild-type female by 2.60 hours. The standard error of the difference being .707 hours, the difference was significant.

The mean duration of life of the ebony male was found to exceed that of the wild-type male by 15.29 hours. The standard error of the difference being .805 hours, the difference was significant.

The mean duration of life of the ebony female was found to exceed that of the wild-type female by 17.98 hours. The standard error of the difference being .85 hours, the difference was significant.

DISCUSSION

In this investigation the mean length of life of the ebony mutant fly under starvation was found to be significantly greater than that of the wild-type fly under starvation. The mutant ebony gene apparently affects not only the coloration of the fly but also the summation of the physiological processes so that the mean length of life increases under the conditions of the investigation. Jennings (1939), in speaking of the work on longevity performed by Gonzalez (1923), says:

As is well known, every gene (with seemingly few exceptions) plays an essential rôle in the life and development of every cell of the body. It is not surprising therefore that changing a single gene may so alter the cellular processes as to change the length of life.

Two explanations for the difference in mean length of life under starvation are possible. Using the duration of life of the imago as an index of its "rate of living" (Pearl, 1928) we may say that the "rate of living" was slower in the ebony mutant than in the wild-type fly under conditions of starvation. It is also possible that the ebony mutant

imago possesses a greater "capital" than the wild-type fly and maintains its advantage throughout life (Ashby, 1930, 1932).

Flies grown under laboratory conditions, where often there are in excess of 200 flies to a bottle, may be considered as under a condition of partial starvation. It may be reasonably stated that those flies which lived longer under a condition of complete starvation would retain this advantage under a condition of partial starvation. As was pointed out in the beginning of this report, the ebony mutant fly was observed to do better under laboratory conditions than the wild-type fly.

Pearl and Parker (1924) in reporting their work on the wild-type fly and its vestigial mutant under starvation used three different densities of population, these being fly densities of 5, 50 and 100 per container. They reported that the mean duration of life of the vestigial mutant under these conditions was much the same as the mean duration of life of the wild-type fly. Pearl and Parker, however, allowed males and females to live together for a short time. Krumbiegel (1929) reported that cohabitation of males and females shortened the mean duration of life in both sexes of *Drosophila*. The mean duration of life of the vestigial and wild-type fly under a density of one might give dissimilar life curves. On the other hand, it is not improbable that the effects of living together vary in different varieties of flies.

The survivorship curves for the ebony mutant fly and the wild-type fly, when placed on a relative time base, were found to be almost identical in shape (*cf.* Fig. 5), although their time placement when computed with regard to actual time is different. Pearl and Parker (1924) found that the form of the life curve under starvation was the same for the fed wild-type and starved wild-type and starved vestigial males. The life curve for the fed vestigial males, however, approached a straight diagonal on an arithlog grid.

The death curves for the ebony mutant and the wild-type fly were skewed to the left (*cf.* Fig. 4). The two

limbs of the curves were very close together and showed a small degree of variation. This gave rather sharply peaked, unimodal frequency curves. Pearl and Miner (1935) state that this type of curve can not be caused by accident. It has been suggested by them that one of the causes for this type of curve is a lethal agent of environmental origin. This lethal agent must be so powerful that it transcends individual variations attempting to nullify its power. Starvation in the present investigation seems to take the place of an externally administered poison. As soon as the endogenous source of energy is lessened to an appreciable extent the toxic substances of the organism accumulate and, after the threshold of toleration is passed, death results. Schlutz, Hastings and Morse (1933) working with mammals reported that inanition may cause the physiological machine to be less efficient for delivering oxygen and removing metabolic products from the tissue.

A study of the survivorship curves of the ebony mutant and the wild-type fly (*cf.* Figs. 2 and 3) brings out a very interesting fact. The last surviving individuals were female flies. Numerous experiments on both man and rats (Bodansky, 1934) have shown that basal metabolism is lowered by starvation. Orr (1937) reported that starvation reduces oxygen consumption in both sexes of *Drosophila*. An explanation of the above observation may be that the basal metabolism of the female decreases more rapidly than that of the male. Thus the "rate of living" of the female would become progressively lessened and as a consequence the length of life increased.

Many workers in the field of longevity have reported that the female is longer lived than the male. Thus Pearl and Parker (1924) state:

The normal relation between the sexes in respect of mean duration of life (females longer-lived than males) observed under full feeding, is preserved under conditions of complete starvation.

It will be recalled that Pearl and Parker worked with flies at densities of 5, 50 and 100 per container. In the

author's investigation, in which the density was 1 per container, it was found that the wild-type male lived significantly longer than the wild-type female. In the work of Pearl and Parker, although the difference in the mean duration of life between male and female favored the female, this difference became progressively less as the density of population decreased. Plotting the differences of the means against the log of the density results in an approximately straight line. This line shows that there is a crossing over in the regions of low population densities and the mean duration of life of the male becomes greater than that of the female. Further experiments are being planned to check the foregoing observations.

SUMMARY

This paper presented the results of the determination of duration of life in 1,550 adult individuals of *Drosophila melanogaster* and its ebony mutant, under a population density of one and conditions of starvation without water. The results obtained were:

(1) The mean duration of life of the ebony mutant was found to exceed in a statistically significant manner the mean length of life of the wild-type fly.

(2) The wild-type male was found, on the average, to live longer than the wild-type female. The difference was shown to be statistically significant.

(3) The mean duration of life of the ebony female was found to be greater than that of the ebony male. The difference was shown to be statistically non-significant.

(4) The last surviving individuals in both the ebony and wild-type populations were females.

(5) The life curves of the ebony and wild-type flies were found to have the same shape when placed on a relative time base.

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SHORTER ARTICLES AND DISCUSSION

SEX-LINKAGE IN PTEROMALUS

DOZORCEVA (1936) has published data from her work with the Chalcidoid wasp *Pteromalus puparum* Linnaeus which are of much interest, as they extend to another superfamily of the Hymenoptera some of the principles of sex-determination demonstrated for the Ichneumonoid *Habrobracon*.

In the latter sex-determination is complementary and a series of multiple allelic sex factors is postulated. Females are heterozygous for sex (xa/xb , xa/xc , etc.) and when unmated produce haploid sons of two types in equal numbers (xa and xb , xa and xc , etc.). When females are mated about two thirds of their eggs are fertilized. If parents are "unrelated," having different alleles ($xa/xb \times xc$), all zygotes are females (xa/xc and xb/xc): if "related" ($xa/xb \times xa$ or $xa/xc \times xc$), one half the zygotes are potentially diploid males (xa/xa , xc/xc). These are highly inviable. Fused, fu , is a sex-linked recessive about ten crossover units from the sex-differentiating factor. In close-crosses of heterozygous female ($xa/xb.fu$) by fused male ($xa.fu$) sex-linkage is apparent, since all four types of zygotes (straights and crossovers) are separable, but in outcrosses ($xa/xb.fu \times xc.fu$) linkage can not be detected, since all zygotes are females.

In *Pteromalus*, red-eye, r , is recessive to wild-type black. Sixty-nine unmated heterozygous females produced males—+ 3,224, r 2,875, giving a relative viability ratio of 0.89 + for red. Mated wild-type by wild-type produced 2,878 females, 3,090 males; mated red by red 102 females, 187 males. Female ratio is therefore 0.475.

Data are not given for crosses of red females by wild-type males, and thus no diploid (biparental) males are demonstrated. However, the similarity of the female ratio, 0.475, to that of inbred lines of *Habrobracon* suggests that many of the crosses may be of "related" parents (close-crosses) with half the fertilized eggs inviable or producing a few diploid males. In outcrosses of *Habrobracon*, female ratio is 0.67 or higher, and there are no diploid males.

Dozorceva obtained progeny from ten matings of heterozygous *Pteromalus* females by red males. Normal sons, 433, exceeded red, 357, because of viability difference as among progeny of

unmated mothers. The ten fraternities fell into three groups as regards eye color of females. One showed near equality—+ 39, *r* 43. One showed marked excess of red—+ 13, *r* 33, while each of the other eight had no red or very few totalling + 270, *r* 22. These three types of fraternities are comparable in respect to red with fraternities in *Habrobracon* involving the sex-linked mutant-type fused. Equality of female types appears in outcrosses, disparity in close-crosses. Sex-linkage is shown by the latter only.

If the ten *Pteromalus* females be $xa.+ / xb.r$ (heterozygous for sex, xa/xb , as in *Habrobracon*), crosses to red males $xb.r$ would give straights—wild-type females, $xa.+ / xb.r$, and crossovers—red females, $xa.r / xb.r$. The diploid males (and inviable zygotes) resulting would be homozygous for sex ($xb.r / xb.r$ and $xb.+ / xb.r$), but these would here be non-separable from their red and wild-type haploid brothers. A cross with red male $xa.r$ would give straights—red females, $xb.r / xa.r$, and crossovers—wild-type females, $xb.+ / xa.r$. The male-producing zygotes homozygous for sex would be $xa.+ / xa.r$ and $xa.r / xa.r$. For eight close-cross fraternities in *Pteromalus*, the crossover females (red) were 22, the straights (+) 270. For one close-cross fraternity the crossovers (+) were 13, the straights (red) 33. Since the mutant-type has lowered viability, the true gametic ratio (recombinations to straights) may be obtained from the square root of the products ($\sqrt{22 \times 13} : \sqrt{270 \times 33}$) from which the crossover ratio is found to be 15.2.

Among the males ratios of red fluctuate near or somewhat below equality, but in one (probably a misprint as the total does not check) it is given as red 12, wild-type 65. The total is given as red 357, wild-type 433, a relative viability ratio for red (0.825) not far from that from unmated females (0.89+).

Sex ratio differs considerably in the different fraternities. In one (♀♀ 12, ♂♂ 266) the great excess of males is undoubtedly due to deficient sperm supply from the mating. The one fraternity giving near equality of the two colors in the females is comparable to outcross fraternities in *Habrobracon*, where sex-linkage is masked. This has the highest female ratio of all (♀♀ 82, ♂♂ 12) suggesting that all fertilized eggs were female-producing and therefore highly viable. Thus this "outcross" in *Pteromalus* may have been heterozygous female $xa.+ / xb.r$ by red male $xc.r$. The zygotes would all be female-producing because heterozygous

for sex (xa/xc and xb/xc) and hence wild-type and red would appear in equal numbers despite sex-linkage.

Greenshields (1939) has considered Dozorzeva's data and has arrived at the conclusion "that it is impossible to derive any of the three categories of results if the genetics of sexuality and eye-color in *Pteromalus puparum* proceed according to the theory of P. W. Whiting." This conclusion is based upon a misunderstanding of my theory. According to Greenshields, half of the eggs are unfertilized by sperm from a given male and are, hence, male-producing; of the other half some are unfertilized and hence also male-producing. If this were true males would always exceed females, but this is contrary to fact not only for *Pteromalus* but for *Habrobracon* as well where females either equal males or exceed them. The misunderstanding probably traces back to my use of the terms selective fertilization, selective syngamy and differential maturation. These terms were used to indicate a selective process occurring *within* the egg *after* the sperm had entered and tending to increase the number of females. According to Greenshields' understanding, sperm would apparently be excluded from half of the eggs, thus tending to increase the number of males. The hypothesis of differential maturation, devised to explain high female-ratio in outcrosses, has now been given up in favor of the theory of multiple alleles.

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THE OCCURRENCE OF DIPLOID MALES IN *HABROBRACON BREVICORNIS*

IN parasitic wasps of the genus *Habrobracon* males are normally produced by parthenogenesis and are therefore haploid. But the occurrence of exceptional diploid males from fertilized eggs has long been known in *H. juglandis* (Ashmead) (A. R. Whiting, 1927), and has recently been demonstrated in *H. pectinophorae* (Fumie Inaba, 1939). It is here reported in a third species of the same genus.

The standard genetic test for recognizing diploid males in *H. juglandis* is made by mating a female homozygous for a recessive gene to a male carrying the dominant allele. Normal haploid sons will show the matroclinous recessive trait, and diploid males can be distinguished by its absence since they develop from fertilized eggs and inherit the dominant allele from the father. Without such a genetic marker there is no certain way of recognizing diploid males except by cytological examination. Diploid cells are larger than haploid ones, and this increment is manifest in such mensurable characters as the number of bristles per unit area of wing surface and the size of eye facets, but these measurements vary so much with size variations among individuals that they are of more value statistically than for particular cases. The importance of the genetic marker to the recognition of diploid males is demonstrated by the fact that when P. W. Whiting reported the discovery of the first good mutant character found in *H. juglandis* he described this anomalous male type in the same paper (P. W. Whiting, 1921). It follows that diploid males may occur in many arrhenotokous species of bees and wasps where the genetics is unknown. Somewhat uncertain cases of patroclinous traits of males have been reported in the honey-bee, but diploid males have not been clearly demonstrated except in the genus *Habrobracon*.

In April, 1937, through the courtesy of A. B. Baird, a culture of *H. brevicornis* (Wesmael) was received from the Dominion Parasite Laboratory at Belleville, Ontario, where it is reared for use in combatting the European corn-borer, whose larvae are its normal host. It is being bred for comparison with *H. juglandis*, similar culture methods are employed and larvae of the Mediterranean flour-moth, *Ephestia kühniella*, which is the normal host of *H. juglandis*, are used for both. Morphologically, the two species are very similar, the chief points of difference being as follows. The antennae in both sexes are longer in *H. brevicornis*; the ground color of body and legs is redder; and the cocoons are longer, frequently are slightly curved, and fit the pupae very loosely.

The Whitings have previously bred *H. brevicornis* and descriptions of anomalous individuals of that species are included in several of their papers which deal chiefly with *H. juglandis* (P. W. Whiting and Anna R. Whiting, 1927; P. W. Whiting, 1932; P. W. Whiting, Raymond J. Greb and B. R. Speicher, 1934). Un-

fortunately, in early papers, before the identity of *H. juglandis* was correctly established, it was erroneously called *H. brevicornis* (P. W. Whiting, 1921). This has resulted in a confusion which still persists, as in a recent paper by Spotkov (Elias M. Spotkov, 1938), where he refers to Hase's work on *H. brevicornis* (A. Hase, 1922), while Hase's stock was actually *H. juglandis*.

Two mutations have been found in *H. brevicornis*. *Defective* causes the complete or partial disappearance of the fourth branch of the radius vein in one or both wings. It is a variable character and does not appear in all the sons of homozygous defective females. It does not reduce viability or fertility and closely resembles the mutation *defective* in *H. juglandis*. *Rough* is also very similar to the mutation of the same name in *H. juglandis*. Wing veins in the region of the fourth radial cell are not straight but irregularly formed with rough edges. In *H. brevicornis* the wings are sometimes extended out at the sides, though this feature does not appear in the majority of rough individuals. Homozygous females frequently produce no offspring, but this is probably due to general weakness rather than actual sterility, as they produce better when furnished host caterpillars previously stung by normal females.

In *H. juglandis* wild-type stocks are easily kept from generation to generation, the progeny of a mated female are allowed to eclose together, sibling matings occur and from these a high proportion of female offspring results. When this method is applied to *H. brevicornis*, however, the female ratio drops rapidly and the stock soon runs to males. This is partly due to the fact that *H. brevicornis* females resist mating, a condition which is now obviated by etherizing virgin females from stock and then placing them with males which readily mate with them.

However, even when females were so mated many of them produced only males. This led to the suspicion that diploid males may be produced in *H. brevicornis*, since in *H. juglandis* they are nearly sterile and females mated to them produce no daughters or very few. Genetic and cytological tests were accordingly made for diploid males. Sixteen rough females mated to type males produced 51 type females and 172 rough males, and in addition to these normal progeny produced 34 type males which were biparental, receiving the normal allele of the rough gene from the father. Cytological examination of these biparental males established the fact that they were diploid, since their

spermatocytes contained twenty chromosomes, while the normal haploid number is ten, as in *H. juglandis*.

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BREAST RIDGE IN DOMESTIC FOWL, A NEW DOMINANT CHARACTER LINKED WITH PEA COMB, OR ANOTHER EXPRESSION OF THE PEA COMB GENE?

WHILE examining a group of dressed carcasses from a number of different breeds and crosses at the divisional killing plant in the summer of 1939, it was noted that all pure Cornish and first-cross Cornish carcasses possessed a peculiar ridgelike formation in the skin of the breast, running longitudinally in the median line just over the breast bone. This "breast ridge," as we have termed it, did not exist on the carcasses of pure-bred Barred Plymouth Rocks, White Leghorns or Light Sussex.

These observations suggested that the characteristic was inherited and led to the examination of living specimens. The findings on the live birds confirmed those made on the carcasses, and it was further found that the characteristic could be readily distinguished on young stock and even on baby chicks.

Fortunately, a number of Cornish crosses and testcrosses (backcrosses) were available and when all pertinent stock was examined it was found that "breast ridge" was present in all pure Cornish,

all F_1 individuals (Cornish \times Leghorns and Cornish \times Sussex) regardless of the direction in which the cross was made and in approximately 50 per cent. of the testercross progeny (Cornish-Leghorn \times Leghorn). These data suggested that the character was a simple autosomal dominant, and additional testercross and F_2 matings were made to test this hypothesis.

In Table 1 are shown the data thus far obtained, compared with those expected on the basis of simple dominant autosomal inheritance.

TABLE 1

Type	Observed		Expected	
	Ridge	No ridge	Ridge	No ridge
F_1	75	0	75	0
F_2	34	11	36	12
Testercross	72	67	69.5	69.5

These data prove quite conclusively that the characteristic "breast ridge" is determined by a single dominant gene. Because the breast ridge is present in all F_1 individuals, regardless of the direction in which the cross was made, sex-linkage is effectively ruled out.

In the fall of 1939, the authors took advantage of the local poultry show to examine a large number of standard breeds and varieties for the presence of breast ridge. The characteristic was observed only in the Cornish, Cornish bantam, Black Sumatra and Brahma (light and dark) breeds of fowl. In other words, it was present only in pea-combed breeds.

The significance of the association between pea comb and breast ridge was not realized until it was noticed in the first F_2 's obtained that all pea-combed birds had breast ridge and all single-combed individuals were smooth-breasted. A re-examination of the test-cross data showed the same association and it immediately became apparent that breast ridge was either, (1) very closely linked with pea comb or (2) another expression of the pea-comb gene. Thereafter, careful observations were made on both breast ridge and pea comb in all F_2 and testercross individuals.

Of the 187 F_2 and testercross birds shown in the table presented in this report, all individuals with breast ridge possessed pea combs and all smooth-breasted birds had single-combs. However, in addition to these, there were three testercross individuals with pea combs which appear to be without breast ridges. In very

young chicks there is occasionally some difficulty in identifying breast ridge, and although one of these chicks is now six weeks old with no sign of a ridge, the association between pea comb and breast ridge has been so consistent, we hesitate to claim that we have secured any true cross-overs. It is possible that our three apparent cross-overs have very faintly expressed breast ridges due to the presence of genes modifying the expression of dominance in this characteristic.

We may conclude, then, that breast ridge is determined by a single autosomal dominant gene located in the same linkage group as pea comb, marbling and naked neck to which Bruckner and Hutt (1939) recently have added the blue-egg gene. Whether this gene is identical with that determining pea comb or very closely linked with it can not be definitely stated at the moment. Further data will be collected which will settle this point in the near future.

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THE BIOLOGICAL BASIS OF SOCIAL PROBLEMS¹

INTRODUCTION

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THE following papers were presented at a symposium held in honor of Professor William E. Ritter at the meeting of the Western Society of Naturalists, held at Monterey, Calif., December 20, 1939. In addition to the papers here published there was an interesting contribution by Dr. H. S. Jennings dealing with the social behavior of *Paramecium*, but it is not included here because arrangements had previously been made for its publication elsewhere. During the same meeting of the society another symposium was held in honor of Professor D. H. Campbell, who for many years has been professor of botany at Stanford University. The idea of holding symposia in honor of these two distinguished investigators who have long been prominently identified with the development of their respective branches of science on the Pacific Coast was a most happy one, and the dedication of a symposium on the biological aspects of social problems to Dr. Ritter was peculiarly appropriate because of his broad human interest in the social import of the biological sciences.

¹ Symposium on "The Biological Basis of Social Problems," held in honor of Professor William E. Ritter at the meeting of the Western Society of Naturalists, Monterey, Calif., December 20, 1939.

A FEW WORDS ABOUT NATURE¹

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It has seemed to me that this meeting of a group of professed naturalists should be a fit time for some brief, easy reflections on what is meant by the terms *nature*, *natural*, *naturalist*, etc. I have lately become impressed by the great variety of usage of these words. What is the meaning of the familiar phrase that such and such an object or act is "perfectly natural," or the almost equally familiar phrase, "Let nature take its course"—common even in economics and business? And what does a philosopher or theologian mean by the "nature of God" or of the "soul" or of the "spirit"?

How many of us here are aware that a few dyed-in-the-wool philosophers, especially in our country, are declaring themselves to be naturalists, even though no one of them seems quite to agree with any of the others? So time-honored, if for no other reason, is *naturalist* as used by groups like this that it would appear only *natural* for us to feel ourselves somewhat competent if not obligated to contribute a bit to the clarification of this bewildering situation.

For myself, I eagerly acknowledge my indebtedness to W. K. Brooks for a suggestion that has grown in significance with me. Brooks is reported on unquestionable authority to have remarked once in a conversation that there seems to be a misunderstanding about the term "supernatural," for, he said, "Nature is everything that is." After much reflection I have ventured to modify this somewhat, though keeping, I am quite sure, to the spirit of Brooks's statement. My modification has been merely to bring the idea into fuller accord with the idea of time. The "is" of Brooks's expression is obviously, it would seem, more restrictive to the "now"—to the "present"—than he, a zoological evolutionist, would stand for. Here is my revision: Nature is everything

¹ Read at a symposium on "The Biological Basis of Social Problems" held in honor of Professor Ritter at the meeting of the Western Society of Naturalists, Monterey, Calif., December 20, 1939.

that is, everything that has been and everything that will be—so far, that is to say, as we have any trustworthy information and understanding.

The main implications of this as to the Natural Sciences is clear. Geography, geology, meteorology and astronomy, also physics and chemistry range themselves at once as natural sciences no less than do botany and zoology; anatomy, physiology and ecology; embryology, genetics and biology. That is all obvious and easy. But what about the vast display of the Humanities—well so named? What about man's language and art; government and other social phenomena; knowledge itself including science; philosophy, ethics and religion? Are all these included in Nature? Here is where the shoe pinches. And the pinch is too tight and difficult for such a nursling naturalist as I am to adjust, especially in a "few words."

Here, then, are the last of my few words:

(1) Reinstate in the idea of biology the meaning it would have if true to the word *bios* with the meaning it had to its originators, the Greeks. This done, the title of the symposium with which you have graciously honored me would be essentially as follows: "That Portion of Biology Which Deals with Social Problems." The slight attention that is given by us moderns to the historic and lexicographic fact that *bios* as used by the Greeks meant "the way of life, especially of men" is strange. For example, "Bios Ellados" was the title of a treatise on Greek civilization written by a historiographer of the later Aristotelian school. This is our illustration from the ancients. Now for a thoroughly modern illustration: E. W. Scripps, certainly one of the brainiest men I have ever known, evolved the idea from his own general experiences, with no coaching from the outside, that he wanted to form a department of sociology in the biological institution at La Jolla, now the Scripps Institution of Oceanography. In accordance with these views hardly anything is more unfortunate for modern culture and civilization than the very prevalent conception that human society is not natural in the same sense that hymenopterous or avian societies are natural. For

seemingly, this conception is largely responsible for the doctrine that social science is not, nor ever can be, natural science.

(2) View nature in this revised Brooksonian fashion; notice also how it is adumbrated by the labors of a few such persons as Aristotle, Confucius-Mencius, Marcus Aurelius, Spinoza, Goethe and above all by Charles Darwin, and you will not (if your experience is like mine) see the slightest ground for conceiving something utterly different from, or outside of, nature as the reality by which we are certainly surrounded every moment of our lives, and upon which our coming into existence and continuance therein are certainly dependent. You will feel no need or desire for some goodness thus exterior to nature, nor any dread or fear of some evil similarly exterior. In a word, this conception of nature leaves, for me at least, no place whatever for such an idea as the supernatural, even though this be garbed in such beguiling terms as metaphysical and metaphysics.

In view of the fact that ethics and religion received special attention as social factors in the symposium, I probably ought to be more specific about these in such a view of nature as I have sketched.

When human nature has been analytically studied as thoroughly as the rest of nature has been and is being studied by the natural sciences, and also synthetically studied as thoroughly as traditional philosophy had erroneously supposed it was studying them, it will be found that the basic factors of both the emotional and rational nature of the human species which make the species moral and religious, should by further development do more for the good of the species than is possible on the basis of any theory or belief in supernatural factors of any kind or name.

To be rid of supernaturalism in every aspect of human life, not by explicitly rejecting it, but by such a development of naturalism that there shall be no longer any need or room for supernaturalism, is the great stage of cultural evolution in which the human species is now struggling.

SOCIAL INTEGRATION AS A BIOLOGICAL PROCESS¹

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It has been debated, sometimes with considerable vigor, whether the social group or unit is to be regarded as an organism. A parallelism has been drawn between the organs of the biological organism and the differentiations within a society or social group and between the division of labor in the organism and society. On the other hand, it has been pointed out that there is physical continuity in the organism, but not in society. It has been maintained, mistakenly it appears, that society has nothing corresponding to the sensory apparatus and the central nervous system of the organism. The psychological factor in human society has often been regarded as rendering any comparison between a society and an organism without significance. Whether we call a social unit an organism or not does not appear to matter greatly. It is, however, a fact of some significance that neither the biologist nor the sociologist is able to avoid use of the term "organization" both as a process and as a condition, and the term apparently has much the same significance for both. As commonly used, it means development or presence of a definite orderly pattern with definite relations of its parts. In the organism the development of this pattern involves a physiological integration of some degree and kind, which makes the organism in some measure a unity. Integration involves development or presence of more or less difference in the parts integrated in both organism and society. Integration and differentiation are both features of organization; integration, either in organism or in society, is impossible without difference of some degree or kind in the parts or individuals to be integrated. The present purpose is merely to call attention to some of the rather striking

¹ Read at a symposium on "The Biological Basis of Social Problems" held in honor of Professor William E. Ritter at the meeting of the Western Society of Naturalists, Monterey, Calif., December 20, 1939.

similarities between physiological integration and social integration.

In the organism two groups of factors are concerned in integration: First, occurrence of energy transformations and transmission of their effects without mass transport of substance from the point of origin to that of effect. The chief of these factors is excitation of living protoplasms and its transmission, either through or along the limiting surfaces of protoplasms, or with more advanced differentiation, by means of nerves. Protoplasmic transmission in the absence of special transmitting organs, the nerves, is usually not very effective, as compared with nervous transmission, and its range of effectiveness is commonly more or less limited. Nervous transmission is more effective, and in the nerves of higher vertebrates its range of effectiveness appears to be limited only by the length of the nerve. The second group of factors concerned in physiological integration is production, transport and effect of particular substances; the various hormones are conspicuous in this group.

Local excitation by some external energy and its transmission can occur without preëxisting difference of the parts concerned. The primary excitation establishes a difference, at least for the time being, and in consequence dominates or controls the parts to which it is transmitted. For a production of a particular substance by a particular part and a particular effect of it on some other part or parts, the two parts must already be different in some way and to some degree. If both are producing the same substance at the same rate it does not become an integrating factor. If one is producing it at a higher rate than the other, that is, if the difference between the two parts is quantitative, there may be some integrating effect of its transport from the former to the latter. The increase in concentration in or about the latter may inhibit production there because of the difference in physiological condition, or perhaps alter the course of reactions otherwise. In any case, it is evident that these material factors, hormones, etc., do not represent the most primi-

tive sort of physiological integration. For that we must look to excitation and its transmission for which no preëxisting difference in the parts concerned is necessary. For localized production and effect of material factors an organization pattern of some sort must already be present. It does not necessarily follow, however, that physiological integration always begins with excitation and transmission.

Essentially the same two groups of factors are concerned in social integration: First, communication, which is really transmission of excitation by one means or another, by voice and hearing, by reaction through vision to behavior of others, to books, to factors in external environment, and so on. The media of social communication are not protoplasmic; they may be air, the telegraph, the telephone, the written or printed word, but they represent transmission, not transport. Moreover, many of them, like the nerves of organisms, are fabrications of living protoplasms.

The other group of factors in social integration is material production and exchange, the commercial factors. As in the organism, these material factors play no part socially until some degree or kind of differentiation or pattern is present. If all persons in a group produce or obtain the same amounts of the same substances there can be no commercial relation between them. If some have more, others less, an integration may result in which the latter receive from the former, but if some are agriculturists, others hunters or fishermen, barter and exchange, regular commercial relations become possible, but these relations depend on communication.

Analysis of these integrative relations in organisms and society shows that they are relations of some degree of dominance and subordination, of control and of being controlled. Both transmitted excitation and transported substances exercise control in organisms. Both communication and commercial relations are controlling factors in social integration. Experiment shows that in many of the simpler axiate animals integration is more or less

completely autocratic in character, so far as development and structure are concerned, that is, control in development is largely or wholly a one-way relation and this control is associated with the physiological activities which we call metabolism. Along the polar axes of such organisms we find evidences of a gradation in rate of metabolism, and the region in which rate is highest is dominant and to a high degree independent of other parts. So far as behavior and structure are concerned, removal of other parts has little or no effect on a hydroid hydranth or a planarian head. The hydranth may finally die from lack of oxygen because other parts are not present to support it free above the substrate and the head may die of starvation, but each remains what it was. Very similar situations as regards autocratic or oligarchic governments cut off from their subordinate populace are not far to seek in human history. In these forms, however, isolation of other parts from the hydranth or head affects both their behavior and structure; as will appear, they are badly handicapped until a new dominance arises and again establishes order.

This autocratic type of dominance in hydroids, planarians and various other forms is usually limited in range of effectiveness, that is, communication is not good. Probably under the conditions it is the only type of dominance that can maintain a fair degree of integration in these forms. However, it is usually not completely successful and physiological isolation, that is, escape from control, occurs frequently in parts most distant from the dominant region. This escape may result from increase in length of the body, from decrease in effectiveness of dominance by weakening, aging or inhibition of the dominant region, by blocking of passage of the effective factor, or by activation of a subordinate part by some external factor to such a degree that it becomes insensitive to the control of the dominant region.

All of these situations are paralleled in human relations. The group may become too large to be controlled, particularly if communication is poor, the autocrat may

become old or ill, his decrees may fail to reach the more distant parts of his domain because of flood, earthquake or failure of the messengers, or local conditions may arise in a less completely subordinated part of the group, resulting in rebellion and a declaration of independence.

A new dominance usually develops from the most active region of the physiologically isolated part, that is, the portion of it which reacts most rapidly or most intensely to the altered situation establishes a new government. Human society affords plenty of parallels for this situation. Except in primitive societies, rebellion, or active separation, is perhaps the most common factor in the social field. In the organism the new dominance is essentially of the same type as the old; there is no improvement in the government. Socially there may or may not be improvement.

As regards initiation and establishment of dominance most eggs give us little information. At the beginning of embryonic development the egg usually has a definite pattern of organization, presumably established during its ovarian history, and in the so-called mosaic eggs this organization approaches that of a well-established social group in which every member knows what he has to do for some time to come. Buds and reconstitution of isolated pieces of the simpler animals bring us nearer to the beginnings of developmental pattern. Here only certain of the reconstitutions need concern us. Reconstitution of isolated pieces of hydroids, planarians, many annelids and various other forms presents certain interesting aspects of dominance which again are not very different from happenings in social groups. In general, whatever the body level from which a piece is isolated, that part of it nearest the original dominant region undergoes activation following the isolation, becomes a new dominant region and induces reorganization in other parts of the piece. This new dominant region becomes the hydranth or the head, but it is more or less dominant before it differentiates, though its dominance may not be fully effective until it reorganizes the means of communication, that

is, the nervous system. In other words, with physical isolation from the original government a new government arises and brings about more or less extensive reorganization in other parts. Here, as in physiological isolation, it is a government of the same kind as the original, and an individual of the same kind results. In the hydroid and the planarian the dominant region is self-differentiating; it can develop in complete absence of other parts. If it fails to develop because of inhibiting conditions, reorganization into a new individual does not take place; without the dominant region the piece can not give rise to anything above the body level from which it was taken; it possesses no initiative and remains only a piece.

Under certain conditions activation at both ends of a piece is sufficient to determine a dominant region at each end. Each of these induces reorganization until it is blocked by the reorganization induced by the other. Here two independent governments have arisen, each exercising control over a part of the piece. It is not difficult to find situations closely paralleling these reconstitutions in social integrations, isolations and reintegrations.

If a dominant region is grafted into some other part of the body it may develop quite independently of its position and bring about reorganization in the body of the host, even sometimes in both directions, distally and proximally or anteriorly and posteriorly from itself for a greater or less distance and in planarians, even when it is the dominant region of another species. In these cases of grafts the reorganization may involve complete obliteration of the original host pattern and reversal in direction of the order distal or anterior to the graft. This approaches a revolution in the parts concerned, but again the new government is of the same kind as the old.

In the course of progressive biological evolution there is approach to a more or less democratic type of organization, but with many halts and digressions on the way. In the mature higher vertebrate and human organism, for example, there is something like representative government. It has often been pointed out that the brain, more

particularly the cerebral cortex, functions as a sort of deliberative body acting on the messages it receives from other parts. The transportative or commercial relations among the parts, hormones, oxygen supply, nutrition, etc., play a role in determining the character of the messages and of the resulting action. Every part of such an organism can, on occasion, get a message through to the central deliberative body and if it is sufficiently important, action results. If something unusual happens to the part, it may become a pressure group, like some social groups with which we are familiar.

The simpler organisms with autocratic dominance show a characteristic particularly interesting in this connection. Any level of the body is capable of becoming a dominant region, provided it can escape from the dominance of another part. The autocratic government has not obliterated the capacity to dominate, if the opportunity is given. The functionally more or less democratic higher vertebrate, however, is, in mature stages, an integration with highly stable structure, like a long established democratic organization in which the class limits have become more or less fixed. The central deliberative body, the cortex, is functionally the most highly labile or plastic part of these organisms: If it ever loses this lability the evolution of human intelligence and, so far as human beings are concerned, social evolution, will probably be at an end. There is considerable evidence that dominance in the earlier embryonic stages of vertebrates, including man, is more or less autocratic in character and that the general structural pattern is largely determined by this type of dominance.

It is perhaps of some interest to note that the more completely communistic organism complexes, consisting of many similar zooids, have remained relatively primitive in character, the chief relation between components being nutritive. There may be dominance and subordination in each zooid member, but there is little or no integration or dominance of the whole.

It may perhaps appear that we have been largely con-

cerned thus far with physiological integration as a social process rather than with the reverse. So far as this is true, it is because this has seemed the simplest and most direct way of emphasizing the similarities of process as distinguished from mechanism. The mechanisms of physiological and social integration are, of course, quite different.

In processes of social integration all types and degrees of dominance appear. Dominance may reside in the personal leader—in the primitive group, the wise man, the old man, or the one who, by virtue or by vice of some characteristic, imposes himself as leader. The most persuasive talker, the greatest bully, the most lawless gangster, may dominate. A particular coincidence of conditions and personal character may determine leadership in an individual who, under other conditions, would have remained an inconspicuous member of the group. Such personal dominance may range from the purely autocratic to a democratic type in which the leader is accessible to all members of the group, may be advised by any or all, and acts or recommends on the basis of that advice; that is, integration in relation to a person may range from the type found in the hydroid and planarian to that of the higher vertebrate.

There is, of course, a psychological basis for social integration. Integration may result from fear of or respect for a person, or personal dominance may have a political or religious basis. But abstractions, concepts, symbols, even verbalisms, are dominant in many social integrations. An institution—the state, the church, the college, the school, the lodge, the club, the general purpose of a particular group—is often dominant as an abstraction, not infrequently as a word, and such dominance may be almost as autocratic as physiological dominance in hydroid and planarian development. In this case, however, the dominant factor in the integration exists only as an abstraction from the group itself, but it is none the less effective. Even the personal leader may become an institution, an abstraction, in the minds of members of the group which he dominates, and as such he may be more

completely dominant than as a person. On the other hand, the dominance of an institution may be democratic, permitting discussion, revision or shift of dominance, and between these extremes all gradations appear. As in the organism, with development of means of communication and transport, the effective range of social dominance increases greatly: The great nation-states with populations of millions spread over thousands of miles of territory are examples.

Is the parallelism between physiological and social integration a mere analogy between fundamentally different activities of living protoplasms? It seems to be more than that. Dominance does not originate autonomously, either in organisms or in social integration, not even when we implant a dominant region into some other part, nor when a personal leader imposes himself on a group. It originates as a reaction to something, as a feature of the behavior of living protoplasms. The basis of both physiological and social integration is the more general and fundamental, perhaps one may say, the more primitive reaction patterns of living protoplasms to physiological, social, or other factors in their environments. These reactions are determined by the potentialities and limitations of protoplasmic behavior. Human reactions are much more complex and varied than those involved in development and maintenance of hydroid or planarian pattern, or even of a human embryo, but they are after all realizations of the same general potentialities of reaction and subject to similar limitations.

If evolution is a fact, rather than an idle dream, similarity of the more general behavior expressions of living protoplasms, whether cells or human beings is no mere analogy, but an essential feature of evolution. Also, if evolution is a fact, the change in character of physiological dominance and integration from the autocratic toward the democratic type may perhaps make us a little more hopeful regarding the future of mankind in the course of biological time, even though the character of dominance in some of the social integrations of the present day is far from encouraging.

THE NATURALIST AS A SOCIAL PHENOMENON^{1,2}

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I PRESUME that I have been included among the speakers in to-day's symposium merely for the reason that I am an old friend and admirer of Dr. Ritter's and not because I am really expected to contribute any ideas of importance on the "biological basis of social problems." Indeed, the title which I first suggested for this discourse was quite a different one from that which appears on our program. It was: "Is There Any Present-Day Need for a Society of Naturalists?" But Dr. Taylor readily convinced me that such a title would not harmonize very well with the general title of this symposium.

Nevertheless, the rhetorical question which I have just set before you springs from an obsession of mine of long standing, and this is such an insistent one that I shall devote a considerable part of my time to the attempt to answer it.

Let us consider first, however, some of the implications of the title actually adopted: "The Naturalist as a Social Phenomenon." The thought at once arises: How could the naturalist, or any other scientist, exist except as a social phenomenon? Leaving out of account the considerable cultural background which must develop before science is possible, it seems evident that the motivation of the individual scientist is necessarily largely social.

We as a group are often credited by idealistic writers, outside of our own profession, with a "disinterested love" of some abstraction called "Truth," or "Science," or what not. Of course we have too much sense to believe that sort of stuff ourselves. But we are, I think, prone to believe that we are actuated primarily, if not exclu-

¹ Read at a symposium on "The Biological Basis of Social Problems" held in honor of Professor William E. Ritter at the meeting of the Western Society of Naturalists, Monterey, Calif., December 20, 1939.

² Contribution from the Scripps Institution of Oceanography, New Series, No. 97.

sively, by our intellectual interest in what we are doing. How far is that really true?

I have confronted myself, and various of my colleagues, with some more or less embarrassing questions, which are not, I presume, altogether original with me. Would you keep on making these interesting observations if publication or any other outlet for your discoveries were denied you? I think that I hear an almost unanimous negative reply to the question. Frankly, it is the reply which I myself should give. I admit that the conditions suggested would be rather rough on you. So we'll let you publish your discoveries in full, but with the proviso that they shall always remain anonymous. No one but yourself would ever know the secret of their authorship. I have a strong conviction that you would still hesitate, and that if you really decided to go ahead, it would be with vastly diminished zest. At least, I may so speak for myself. Nor is this necessarily a confession of vanity or selfishness. It is merely another bit of evidence that even *Homo scientificus* is a gregarious species. Our satisfactions are derived largely from the approbation and sympathetic understanding of our colleagues, from the interchange of ideas and—in very varying degree, it is true—from the belief that this hard-won increase in knowledge is, on the whole, a good thing for mankind. This last assumption is, of course, the only justification for the public support of scientific work, and I believe that the assumption is fully warranted. But the degree to which the individual scientist is motivated by such considerations is certainly highly variable. It is only too evident that in extensive areas of the world at present, important fields of science are being exploited for purposes quite other than human welfare. However, we naturalists may take satisfaction in the knowledge that none of the discoveries which we make in our field lends itself very well to the butchery of our colleagues who speak some other language.

What, then, is the peculiar field of the naturalist? My

past remarks, for the most part, would apply equally well to scientists of any sort. I shall not bore you with any attempt to define this term. Several quite different meanings are attached to the word, as will be found by glancing into a dictionary. What I mean here by a naturalist will develop in the course of the ensuing remarks.

I fear that the word is one which too often arouses feelings of antipathy or contempt in the mind of the modern biologist. It is difficult to escape the popular caricature: a wizened, bespectacled individual, who spends his time sorting and naming pickled specimens or endless rows of insects—an eccentric whose chief outdoor diversion consists in sweeping the air with a butterfly-net or gathering snakes and spiders in his wife's cooking utensils. At best, we are apt to think of a naturalist as an unfortunate who has not been able to keep up with the procession of those who are making the real advances in biology. Indeed, we are wont to prefix the words "old fashioned" when we apply this term to anyone.

What, then, is a naturalist? To say that he is a person who concerns himself with natural history would not, I am afraid, be very helpful in clearing our friend's reputation. For the expression "natural history" even more suggests something altogether primitive and out-of-date—a pursuit suited only to children and amateurs. Indeed, we learn from "Webster's Dictionary" that this term is "now commonly restricted to a study of these subjects [zoology and botany] in a more or less superficial way, at least without making use of modern anatomical and analytical methods." And a glance at the endless popular volumes carrying the title of "natural history" would seem to bear out this statement.

Are we, however, forced to accept such a depreciation of a term which has had such long and honorable associations? Listen to the words of one of our greatest naturalists, William Morton Wheeler:

History shows us that throughout the centuries, from Aristotle and Pliny to the present day, natural history constitutes the perennial root-stock or

stolon of biological science and that it retains this character because it satisfies some of our most fundamental and vital interests in organisms as living individuals more or less like ourselves. From time to time the stolon has produced special disciplines which have grown into great, flourishing complexes. . . . The stolon relationship of natural history, or ecology, to the other biological disciplines is of great theoretical and practical significance. Nearly all the important biological problems, especially of a physiological or morphological character, have arisen in the course of simple investigation in natural history and many of the more difficult of them have been turned over to the special disciplines for solution. On the other hand, the ecologist is continually drawing on the methods and resources of physiology, morphology, taxonomy, distribution, etc., in solving his own particular problems of adaptation and behavior.

It is evident that Wheeler's use of the word "ecology," in this connection, is considerably broader than that given it by the societies and journals which bear that name.

Wheeler's metaphor of the stolon seems to me admirable, though it is defective in one respect. For the individual plants or animals which are budded off from a stolon are capable of detaching themselves from the parent stock and becoming independent organisms. Our specialized branches of biology, on the contrary, can not live independently of the parent stock and in so far as they attempt to do so, they languish. (All this Wheeler himself would, of course, have insisted upon.)

We may state this last idea differently by stressing the importance of maintaining the natural history point of view, whatever specialty one may happen to be pursuing. Regarded from this angle, we might consider anyone to be a naturalist who consistently maintained the natural history point of view. This is the point of view which looks beyond the immediate object of one's pursuit and does not lose sight of its relations to a larger whole. It stresses synthesis as a necessary complement to analysis, integration as a necessary complement to differentiation.

The naturalist, as thus conceived, never forgets that he is dealing with organisms dwelling in environments, even though he may be concerned for the moment with cells, chromosomes, genes, sols, gels, ions, unsaturated valencies or what not. He recognizes the autonomy of biology as a science, and does not lose interest in those activities

of organisms which he can not subject to physical or chemical analysis. In general, I think that he will subscribe to the dictum of Dr. Ritter that "the organism in its totality is as essential to an explanation of its elements as its elements are to an explanation of the organism."

I am tempted to add that in the field of taxonomy the naturalist, as thus described, is more likely to be a "lumper" than a "splitter," though I confess that this is merely a wild guess, based upon my notions of the fitness of things. For the "lumper" is a person who recognizes the importance of stressing resemblances as well as differences, of synthesis as well as analysis.

Do I seem to be wandering too far from the subject-matter of this symposium? I think that I am hewing to the line. What can be of more importance socially than the integration of our knowledge—the assimilation of each new discovery to the past acquisitions of our race? On the contrary we may well query whether extreme specialization may not at times become an anti-social process. When it loses its sense of perspective and adopts an esoteric vocabulary and ideology, does it not actually become an eddy moving in the wrong direction?

Even these references to taxonomy really have a very distinct social slant, as I was long ago moved to point out. For the unchecked splitting of genera results not only in creating confusion in the terminology which we are all forced to use, but it destroys what we may call the "verbal clues to the nearer kinships between species." This situation handicaps a group much wider than that of the professional biologists.

It may be pertinent at this point to recall the question which I put before you at the outset: "Is there any present-day need for a society of naturalists?" I do not have in mind merely a question of name. I have in mind the subjects to be discussed by such an organization, and more yet the manner of their treatment. We have our special societies, which now represent almost every minutest subdivision of the life sciences. It is not my object to deplore

the specialization here implied. This seems to be the price which we must pay for advance in science.

Should there not remain, however, a few organizations which specialize, so to speak, in the more general aspects of biology; which maintain consistently the naturalist's point of view? A society is at least conceivable having as its primary object the presentation of programs calculated to be intelligible and interesting to all members of a diversified audience such as the present one. By this I do not have in mind providing popular programs for the laity. That is quite another problem. I am thinking of ourselves. I have in mind the need among workers in diverse branches of biology, for cultivating and extending such common ground as still remains to us in spite of our increasing specialization. It would not be possible or even desirable, to limit the programs of such a society to discussions in the field of general biology. Reports of individual researches might be welcomed as usual, but with certain very important restrictions. The society should insist that every paper admitted to its program must rise above the merely descriptive level, and that its author must make it clear why we should be interested in what he is telling us.

Or is this a counsel of perfection? Perhaps it is. At least, I have often been told so. And yet I am so convinced that the contrary is true that I have bored some of my colleagues for years with my protestations. Not that these persons do not admit the deplorable condition of so many of our scientific programs, but they adopt a defeatist attitude and argue that the situation is incurable.

One is inevitably met with the challenge: How many of our biologists, particularly our younger ones, are capable of interesting a group as diversified as this? I fear it is true that sufficient attention has never been devoted to training our graduate students to be interesting. Since most of them are merely going to be teachers, perhaps this has not been regarded as important. However, I think it might have a salutary effect if we admitted to our an-

nual programs only those who have made some progress in the art of being interesting. Let the impression get around that it is an honor to present a paper at one of our meetings, and that only the more competent men can make the grade. The practice in the past has too often been to accept with thanks anything that is offered. Is not the alternative plan worth more serious consideration? It might shorten our programs, perhaps, but would that be an unmixed evil?

This matter of being interesting includes, of course, something far more than mere literary or rhetorical skill. It includes the ability to see the broader implications of one's discoveries and to present them intelligibly to persons who are not fellow-travelers in one's own particular compartment. If these broader implications are not discernible, the paper does not belong on the program of a society of naturalists at all. There are more highly specialized organizations before which such communications may be presented.

I must add that these later remarks of mine have a much broader application than merely to our hypothetical society of naturalists. The criteria of interest and intelligibility should be applied even to our more narrowly specialized programs. That these criteria are not sufficiently adhered to has resulted in a situation so often expressed in the declaration: "I don't attend the meetings to listen to papers; I go to visit with my friends." Such an attitude is unduly pessimistic, I think, but we must admit that it has considerable justification.

Regarding the kind of material appropriate for the programs of a society of naturalists, I fear that my remarks thus far have been rather indefinite. Suppose that we get down to cases. It is naturally much easier to pick out examples of things that do not belong on such programs than it is to offer helpful suggestions along constructive lines. This because the pages of our technical journals are filled almost exclusively with material of the former class. Here are a half dozen titles which I jotted

down a few days ago from the contents of recent issues of an equal number of leading American biological journals. They are, I think, quite representative ones. As contributions to the literature these papers are all, perhaps, impeccable. But my hypothetical censor would exclude every one of them from the program of a society of naturalists. It is even possible that an intelligent and sufficiently hard-boiled censor would rule that some of these were unsuited for presentation to any audience comprised of more than six persons. Here are the samples:

An analysis of the chromosomes of the two subspecies *Drosophila virilis virilis* and *Drosophila virilis americana*.

A new race of sharp-shinned hawk from Mexico.

Activity-preventing and egg-sea-water neutralizing substances from spermatozoa of *Echinometra subangularis*.

The functional significance of the capillary bed in the brain of the opossum.

Effect of the introduction of the nitro group into the phenol molecule on toxicity to goldfish.

Experimental study of the function of the corpora allata in the grasshopper *Melanoplus differentialis*.

This is no carefully selected list. It represents a fair sampling of the titles which make up the tables of contents of these various journals. They reflect the prevailing modes in biological research. They all probably represent profitable lines of research.

To pick out an equal number of papers to illustrate the kind which I believe to be suitable for presentation before a society of naturalists is much more difficult. Practically nothing of promise in this direction is to be found in any of the six journals from which the preceding list was taken. One thinks at once of the AMERICAN NATURALIST in this connection. But for years the contents of this journal, with the exception of the symposia which it publishes, have been for the most part highly specialized. Many of the contributions might equally well have appeared in one of the journals of genetics. It is interesting to compare the successive volumes of this important periodical from its inception in 1868 to the present day. We have a pageant of the history of biology in America. It is a long way from Morse and Packard and Hyatt and

Cope to Morgan and Jennings and Child and Parker. Nor can we deny that this trend, on the whole, represents progress.

The sort of material that we are looking for now is best exemplified in the subject-matter of some of the symposia, published in the *AMERICAN NATURALIST*. (I may add, in the subject-matter of some of the symposia of our present meeting.) Such topics as "Adaptation," "Temperature and Life," "The Species Problem," "The Biology of Sex," "The Cell Theory" and "Contributions of the Higher Animals to an Understanding of Human Biology" surely belong to the realm of natural history in the sense which Wheeler had in mind.

I think none of us, however, would wish to restrict our programs to symposia. Single contributions, invited or volunteered, which meet the requirements of relevancy would probably continue to furnish the backbone of the program.

Since I have said so much about relevancy, it seems a fair request that I should cite the titles of some papers which I should expect our naturalist-censor to admit to his program, though it is obvious that the title alone would not guarantee the acceptability of a paper. With some hesitation I offer another list of six titles which I jotted down after a much more prolonged search than that required for the first list. It would have been a much simpler matter to invent a series of such titles, but it is my wish to restrict myself here to actual ones:

Biological effects of population density in lower organisms.

Genetics of quintuplets.

The toleration of solar heat in desert reptiles.

Homing instinct in salmon.

Facts and theories of bird flight.

Survival value of acridian protective coloration.

It happens that this list is predominantly ecological in its complexion. But this was because the majority of available titles which I encountered were ecological ones. I do not see, however, why discussions based upon the

data of almost any one of the recognized sub-divisions of biology should not be relevant to a naturalists' program if properly handled. But the physiologist should not address himself primarily to the physiologist, nor the geneticist to the geneticist, nor the embryologist to the embryologist. Each should phrase his discussion as little as possible in the peculiar vocabulary of his specialty, and should make a determined endeavor to keep in view the broader significance of the data which he reports.

In fact, I seriously question whether such an organization as we have been discussing should be conducted primarily as a publicity channel through which its members may report their very latest series of experiments or observations. If it is really our object to be mutually informative, would not this object be far better achieved if we presented carefully digested reviews covering the general fields in which our particular research problems lie? Could not the detailed reports of progress usually be better left to our published accounts in the journals? Must we really lose caste by serving for the moment as teachers of one another?

Is all this sort of thing possible, or is it just another "Ham-and-Eggs" proposal? While I am not offering any precise mechanism by which these beneficent results could be achieved, I believe that they could be achieved if we really took the matter seriously. It would, perhaps, require the same degree of ingenuity that we all so cheerfully devote to our experimental problems in the laboratory—also, a considerable amount of hard work.

The essence of the plan, of course, is the selection of material for the programs. I confess that the picture of a hard-boiled censor is not a pretty one. I am afraid that he would not last very long in an American scientific society. But some benign selective agency is necessary for the purpose of soliciting appropriate material and rejecting what is inappropriate. Why should it be any more ruthless for a qualified program committee to reject a paper from its program than for the editors of a journal

to reject an unsuitable manuscript offered them for publication? Why indeed, could not this committee assume some of the friendly advisory functions which our editors perform in relation to the contributions offered them? There is often a third alternative besides outright acceptance or rejection.

I am quite aware that the line between topics which are "relevant" and those which are "irrelevant" to a given program must, at times, be very difficult to draw. Particularly is it difficult to judge by the title of a paper. I have in mind one rather recent case in which a man offered a contribution in the field of ichthyology, which turned out, when delivered, to be little more than a fervid eulogy of the government of Soviet Russia. Our censor had momentarily relaxed his vigilance. It seems reasonable to require that a completed manuscript, or at least a detailed summary of a paper, should be submitted in advance, as indeed is often done now.

Finally, it must be admitted that less depends upon the topic chosen than upon the way it is treated, in other words, upon the mental outlook of the speaker. One of the most harassing program numbers that ever drove me from a lecture-room was delivered by a contributor to an important symposium, a man apparently chosen because he was an "outstanding authority" in his particular specialty, but with no regard for his sense of proportion or ability to rise above the level of particulars. Here again, our censor had gone to sleep at the wheel.

On the other hand, a speaker who views his subject *sub specie aeternitatis* can make almost any bit of information illuminating. Such are our great lecturers in biology, men of the caliber of Edmund B. Wilson and G. H. Parker.

I think that you will agree with me that a naturalist who can bring to pass the beneficent things that I have been talking about will be not only a social phenomenon but a most valuable social asset. I do not wish to imply that we have not already made considerable progress in that direction, but I think that we still have a long distance to travel.

THE ETHICS OF ENMITY IN SOCIAL EVOLUTION¹

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IN the chapter of his "Principles of Ethics" on "The Confusion of Ethical Thought" Mr. Herbert Spencer clearly showed that throughout human history mankind has followed two diametrically opposed codes of conduct which he calls the "ethics of amity" and the "ethics of enmity." To many persons the expression "ethics of enmity" may seem to imply a contradiction in terms. We commonly associate amity with goodness, and enmity with evil. The mere suggestion that enmity has a useful function, with an ethics appropriate to it, is apt to arouse at once an inimical reaction. Nevertheless, probably ninety-nine out of every hundred who profess allegiance to an ethics based exclusively upon amity would, if their country were invaded by a hostile army, pay scant attention to the maxim "Resist not evil," and would wholeheartedly and vociferously support measures for inflicting death upon their adversaries. They would do this, moreover, in obedience to the dictates of conscience and in the conviction that failure to support the cause of national defense would be base and highly blameworthy.

Up to, and in some countries within, recent times dueling, as Spencer points out, was supported by the strongest moral sanctions, and a man who failed to challenge one who insulted him was looked upon as a coward and a poltroon. Our code of honor befitting a gentleman has changed. We are no longer under a moral obligation to fight duels, but on occasion collective fighting meets with almost universal approval. Nearly every one would concede without hesitation that the Belgians were morally justified in forcibly resisting the ruthless and unprovoked invasion of their country in 1914. Under the circum-

¹ Read at a symposium on "The Biological Basis of Social Problems" held in honor of Professor William E. Ritter at the meeting of the Western Society of Naturalists, Monterey, Calif., December 20, 1939.

stances, according to the recognized moral standards of mankind, right conduct for the Belgians called for the employment of the ethics of enmity rather than amity toward their foes.

In treating of the ethics of enmity I am concerned not with what conduct is right in the best of all possible worlds, but in the present very imperfect conditions of human life to which we perforce have to adjust ourselves. If we are in the midst of a war that has been unjustly inflicted upon us, it will profit us little to declare that war is an evil and that it is wicked to engage in it. We may concede that war is a great evil and that nations should endeavor by all honorable means to avoid it; but when a country is in the throes of a life and death struggle its citizens must adjust themselves to a real emergency. If I were attacked by a pack of wolves I would feel that it would be quite ethical to kill as many of these animals as possible. From the standpoint of the wolves cooperation in the common cause of attacking my person is highly commendable behavior, the kind of behavior which nature has compelled these animals to follow under penalty of dying of starvation. Since I am under an equal necessity of resisting the wolves, we both espouse the ethics of enmity in our dealings with one another.

When we look out upon the world of living creatures we find numerous instances of different species whose interests are essentially antagonistic. The brown rat and the black rat are keen competitors, and now the one and now the other prevails. Man is attacked by numerous parasites and other maleficent creatures which we deem it quite proper to treat as enemies. We would bestow the highest commendation upon any one who succeeded in exterminating the hookworm, the blood fluke, or the pallid spirochaete. But while we should not feel the slightest qualms about killing off myriads of these inimical organisms, there are numerous people who believe that our conduct would be morally wrong, that "all life is sacred" and that no living creature should be wilfully put to death.

Numerous Brahmans, Jainists and Buddhists of India sincerely endeavor to extend the ethics of amity to all living creatures whatsoever, and many thousands of them die annually from the bites of cobras and other venomous snakes which fail to appreciate the considerate treatment accorded them by their human associates. The costly practices of these Hindu sects are the consequences of simply carrying out the ethics of amity to an extreme though logically consistent degree.

As species of animals go, man can not be said to suffer from any lack of instinctive pugnacity. The trait exhibits itself from the earliest squallings and kickings of infancy to the most advanced age. In the course of his long evolution man's nature has been molded by strife and for strife. Nature has made him a fighting animal. For countless centuries he has carried on an intermittent warfare with members of his own species. His religious beliefs, his ideals and his moral codes give evidence of the prominent place that combat has occupied in his life and thought. His literature is filled with the records of heroic deeds. The "Iliad," the "Odyssey," the "Aeneid," the epics of the Norsemen recount the glorious achievements of conquering heroes, and "Paradise Lost" translates the field of battle to the next world, where it is carried on against the powers of darkness by winged hosts of angels and archangels under the supreme command of the Son of God, with Gabriel and Michael contributing powerful support as members of the general staff. Men like to read about fighting, to see fighting, to play at fighting, as well as actually to engage in combat.

If we ask how man came to be constituted in this fashion, the evolutionist comes forward with a plausible answer. It is an answer very different from those formerly given, which attributed man's bellicosity to the animal side of his nature, the machinations of evil spirits, or the total depravity arising from original sin. The Darwinian evolutionist looks upon man as owing his traits to their survival value in the struggle for existence.

Group pugnacity, as well as the instincts leading to mutual aid, he regards as having been evolved because they contributed to the success of the groups in which they were best developed. Wherever societies have been evolved in the animal kingdom there we find an unusual development of both mutual aid and group pugnacity, as is strikingly illustrated among the ants, termites and the social bees and wasps, in contrast to most non-social insects. These seemingly opposed traits are not really opposed, but complementary. In sub-human ethics the conduct to which they lead is good from the standpoint of the species concerned. Animal ethics is therefore in part an ethics of amity and in part an ethics of enmity. So also is human ethics throughout most of human history, and for essentially the same reasons. If we consider the moral codes of almost any primitive people we shall find that there is one code for consociates and another for aliens. In Deuteronomy, for instance, the Children of Israel are given the following command: "Ye shall not eat of anything that dieth of itself; thou shalt give it unto the stranger that is in thy gates that he may eat it; or thou mayest sell it unto an alien."

Illustrations of the ethics of enmity are everywhere in evidence in the approved codes of nearly all peoples. In the Old Testament they occur in the greatest profusion. During their sanguinary struggles the Children of Israel not infrequently slaughtered not only all the men, but the women and children whom Jehovah delivered into their hands. They gained victories right and left because of the supernatural aid they received in their ruthless wars of exploitation. Theirs was the ethics of a primitive and warlike tribe. Their ethics of amity formulated in the Ten Commandments and the directions for the conduct of life set forth in Leviticus, Numbers and Deuteronomy form a sharp contrast to their ethics of enmity which were exhibited in their treatment of the surrounding peoples whose lands, goods, and women they desired to appropriate.

There is nothing essentially unique in the ethics of the ancient Hebrews. Similar dual codes of conduct are found in hundreds of groups of predatory nomads the world over. As primitive tribes expanded or were consolidated into nations the same dual standards, although often less clearly distinguished, have continued in practice. They are deeply intrenched in the thoughts and customs of all the nations of the earth. Notwithstanding the influence of the great religions of Confucianism, Buddhism and Christianity which have held up ideals of conduct that make no distinctions of nation or race in the duties they enjoin, the ethics of enmity still meets with almost universal approval in practice, even among those who condemn it in theory. The present wide-spread hostility of man to man is the product of powerful forces which our increase of culture and humanitarian spirit has proved powerless to prevent. There can be no doubt that war is now one of the greatest evils of the world. There can also be no doubt, I believe, that strife has played an important part during the evolution of man in molding human nature into conformity with the needs of social life and in making man a moral animal.

As a result of social evolution, and especially the advancement of science, the conditions and effects of war have greatly changed. Instead of promoting the evolution of the human species, war is now a powerful means of undoing the work of civilization. That there is altogether too much enmity in the world is a statement about which there can not be the least doubt. But at the same time it can not be doubted that there are occasions in which we are morally justified in treating other human beings as enemies. If a vessel is attacked by a gang of pirates who, if successful, would probably rape the women and make the rest of the crew walk the plank, the course of right conduct would be to repel the invaders, even if it involved killing the entire outfit. And there are many other situations in which right conduct requires the injury or destruction of other human beings. When an individ-

ual is antagonistic to society, society has a certain justification in being antagonistic to him. Formerly the ethics of enmity was almost exclusively followed in the treatment of offenders. Now efforts are made to reform the criminal and thus to minister to his welfare as an individual. But although reform is a highly commendable aim, the welfare of the individual can not be allowed to take precedence over the interests of society. The welfare of the late Mr. John Dillinger might have been enhanced by allowing him to go scot free to continue his career of killing; but society is justified in checking such a career even at the cost of the well-being of the offender.

In one form or another the ethics of enmity still has a legitimate part to play in the promotion of the general good. This arises from the fact that the relations of human beings, like those of the lower animals, are often antagonistic. We may deplore this fact, but we happen to live in this kind of a world. We gain nothing by closing our eyes to this situation. In fact, we are handicapped in dealing with several moral problems by our failure frankly to recognize the legitimate place that the ethics of enmity still holds in our social life. Most of us give lip service to an unqualified ethics of amity and approve the ethics of enmity on the ground of practical expediency. So long as we preach the one and practice the other will our efforts at moral reform fall short of their full possibilities of good. Our common thinking on the topic before us is hopelessly muddled. This is largely due to the fact that we try to avoid seeing our moral inconsistencies.

The ethics of amity and the ethics of enmity should differ only in their proximate, but not in their ultimate, aim. Both should be concerned with the promotion of human welfare in the highest and best sense of this term. How human welfare may best be promoted is a scientific problem and often by no means an easy one. To solve it aright we need to understand the springs of human action and to be able to explain why human beings go wrong.

In order to cope with human disease we should know how diseases are caused, and why. Likewise, it is only through the knowledge of causes in the sphere of morals that the moral physician will be most successful in affecting cures. At times he will have to employ the ethics of enmity to effect a desired reform as at times a surgeon must apply the ethics of enmity to an offending member by cutting it off. The business of moral reform is one that requires all the scientific knowledge that we possess, and more. It can not be done by simply preaching the duty of brotherly love.

With all our progress we have met with little success in coping with several moral problems. In our dealings with two evils especially, crime and war, we have made almost a complete failure. It is quite clear, I think, that we can not hope for much by simply telling individuals or nations to be good. The reformer must delve deep into the springs of human action and endeavor to understand the causes of anti-social behavior among nations as well as individuals. It is only through a changed human psychology that much can be effected in dealing with either of the evils mentioned, to say nothing of many others. We live in a world in which the ethics of enmity is practiced to a degree out of all proportion to its real need. The resulting evil thus caused is in no way mitigated, and may even be considerably increased, by the fact that the ethics of enmity is followed with entire moral approval and devotion to duty.

Through the action of social forces people are sometimes caught in unfavorable situations from which extrication is very difficult. At present this is true in our economic life, as it is true in relation to war. In both a shortsighted and misguided ethics of enmity is a powerful factor in thwarting our efforts to mitigate these evils. We have altogether too much enmity not only because of the important role which enmity has played in the past, but because of the real need for a certain amount of it at the present time. As the day when the lion and the lamb

will lie down together is not apt to arrive so long as the lion is so constituted that he must either feed upon lamb or die of starvation, so the day when swords will be beaten into plowshares will not arrive as long as there are aggressor nations which pursue their course of domination in utter disregard of justice, liberty and the most solemn promises. No, we are not ready to dispense with the enmities that are still necessitated by the good life. On the contrary, an ethics of enmity adapted to present conditions of civilization and generally agreed to is one of the great needs of the world. In his day the learned Grotius went far toward formulating such a system in his great book on "Rights in War and Peace," but notwithstanding the many attempts to regulate the conditions and to mitigate the barbarities of warfare, the present wanton killing of non-combatants, the disregard of treaties and the rights of neutral nations, the inhuman treatment of conquered peoples, compel us to admit that there has been scarcely a period since the days of early savagery when the ethics of warfare has fallen to so low an ebb.

That the present turmoil is highly disadvantageous to all parties concerned admits of no doubt. The elimination of war has to be a cooperative undertaking which all nations sincerely desire to accomplish. The problem could have been solved long ago had statesmen, with the approval of their nations, seriously and sincerely devoted themselves to the task instead of continually scheming and maneuvering by devious methods to secure some advantage over their rivals. There is little prospect of much improvement in international relations until we have developed the arts of cooperative, instead of competitive, statecraft. For this involves much more than a changed attitude on the part of leaders whose policies are largely a reflection of popular sentiment. The cure for the evils of our misguided ethics of enmity will come but slowly and only as a result of changes wrought in the outlook and attitudes of people in general. To this end the

greatest aid is to be expected from the sciences that will give us a better understanding of the nature of man and the forces that determine his behavior.

I have ventured to comment upon the ethics of enmity because it is the one aspect of ethics which is in greatest need of clarification. We only add to the confusion by trying to cajole ourselves into thinking that it has no legitimate place. We need to put it in order; to understand its course of evolution and the reasons therefor, and to define clearly its scope and present limits of application. Under present conditions of the world it is, I believe, in this field that a scientific ethics will find its greatest opportunity for contributing to the welfare of mankind.

A BIOLOGIST'S APPRECIATION OF RELIGION AS A FACTOR IN SOCIAL EVOLUTION¹

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RELIGION is a body of belief accepted by faith, not founded on the experience of the senses. As an element of importance in the history of humanity, it has always included rules of personal conduct. Faith is not in its nature responsible to reason, and religious people have properly denied the right of reason to try to test its articles; more than this, they have often resented such attempts as impious. Freedom of thought and freedom of speech are fundamental American rights. Nevertheless, no longer ago than my memory reaches, freedom of speech on matters of religion was under fairly strict social control, on the reasonable ground that the cherished beliefs of our neighbors were entitled to respect and consideration.

A biologist, as understood in this talk, is one whose scientific work is based on the principle that what can survive survives, at the necessary sacrifice of what can not survive. This is self-evident, to us. But it is the biologist's application of the principle to man that has made it the most distinctive basis of thought during the last eighty years.

The consciousness that man is an animal must have been latent in all humanity. At the same time, man was, and is, the greatest mystery he knows. The sunrise and the thunderbolt have long been explained, sufficiently so that it is the wise, not the ignorant, who still see anything unexplained in them. But man himself remained almost as unexplained as ever, in the general thought of humanity, an insoluble mystery before which the study of law in the universe ceased to be effective. By fixing man's

¹ Read at a symposium on "The Biological Basis of Social Problems" held in honor of Professor William E. Ritter at the meeting of the Western Society of Naturalists, Monterey, Calif., December 20, 1939.

place in zoology, science broke down the greatest barrier to his appreciation of the universal play of cause and effect.

The same general laws responsible for the origin of man have continued to operate on him. Biologists are, of course, concerned with the evolution of later man from his human forebears, but in this study they find themselves on common ground with students of various other kinds. Man's prime initial asset was his intellect, the best in nature, which enabled him to survive and gradually to assume and maintain dominance over the rest of the animate world. During the rise to dominance, selection was focussed on his intellect, because this function was chiefly responsible for his survival. Whenever selection is thus focussed on one function and the correlated structure, they evolve rapidly, as such processes go in nature. I suppose that before the majority of the surviving races of man were differentiated, and before existing man spread over the globe, his intellect reached what we may be pleased to call its present state of perfection. During this early period his conduct, of course, conformed to rules, which favored his survival as an individual rather than as a member of society.

Once man's dominance was secure, his competition for survival was with other men; and in this competition, the intellect of the individual is no longer the most valuable asset. Groups of men could survive in competition with individual men. Thus the social qualifications which enable men to cooperate in stable groups were more important in determining survival than were any minor chance differences in intellect. Herd instinct is a social qualification. The intellect is not. It is individual, in the long run anti-social. If men think together to-day, and are thus united, they are prone to think each for himself to-morrow, and to be disunited, even hostile. On the other hand, just as instincts held together the groups of man's predecessors in whatever measure was good, so man's feelings, partly instinctive and all in part instinc-

tive, have always performed this service for him, even in opposition to the intellect. And his beliefs, more akin to feeling than to knowledge, have always been more effective in this respect than have the intellect or its products, even wisdom.

Let us begin again with most primitive man. He had always an idea of cause and effect. Just as far as his equipment of knowledge let him, he explained phenomena by their causes. So far, the world was natural. But all about him were phenomena he could not so understand. In the absence of understandable, natural causes, he postulated supernatural causes, or agents. These were his gods, many or few, to be placated, eventually, in man's presumption, to be controlled, if only by the manner of his submission. The field recognized as orderly, in which everything was natural cause or effect, grew steadily through the ages, but always permeated and surrounded by the mysterious work of the gods.

Men's common beliefs about their supernatural enemies and eventual friends held men together in groups. Moreover such beliefs involved rules of conduct to placate and appease the gods and particularly to avoid offending them. The resulting common conduct united the believers the more firmly. As the groups with common belief grew larger, and as superstition was restricted and refined and became more worthy of the name of religion, its hold on men gained steadily in strength.

From the beginning, and with ever increasing intensity, men's bodies of belief, their religions, have been in competition for survival. Leaving out of account authority for the belief, the reasonableness of the belief and the truth inherent in the belief—matters which it is not my business to appraise—two elements have determined survival in the competition between beliefs, eventually between religions. These are their effectiveness as social cement, binding and holding men together for common action, and the essential goodness of the associated rules of conduct, their respective moral codes.

If belief were weak or uncertain, it could not hold its adherents during stress; the group must disintegrate even if its members survived; and the belief must disappear. Half-accepted belief has no value at all. But whole-heartedly accepted religion has been the strongest social cement in human experience. Its only rival has been race-loyalty, patriotism, and the two have commonly gone together, the tribe, race or state having its own religion.

Conduct determined survival long before religion began, and still does so. The intellect can appraise a moral code, and find its articles good or bad as their observance is favorable or unfavorable to the prospect of survival. If any religion enjoined practices unfavorable to survival, its believers sooner or later died and the religion disappeared; that has occurred countless times. Naturally, the moral codes of successful religions are much alike, and much like the code successful races would have had to observe in the absence of religion.

While the codes of the biologist, the statesman and a great religion may, and in a measure must be alike, religion has an incalculable advantage in their enforcement. To the individual, religion sanctions its commands with the prospect of eternity in heaven or hell, compared with which a grain of sand hangs in the mouth of the hour-glass while the rewards and punishments of the biologist and of the state run their course. The latter present death as their maximum penalty; but it is as certain for the good as for the bad; religion offers it to the virtuous as the door to an indescribably happier life.

In the brief period of human history, as must have been so in the long period before formal history, religions have continued in competition, and a few have survived and spread because they held men together effectively and their moral codes were fit to survive. Others, even in our experience, have taught bad practices, or practices which with changing conditions became bad; and the evil has been transient, because their badness condemned such

religions to extinction along with their devotees. That is the biological meaning of badness. Good moral codes preserved those who obeyed them and with them the religion of their belief.

This is too simple to need any elaboration before this audience. It is apodictic doctrine, of the type we frankly seek. It is accepted by the historian, the sociologist, the economist, by the body of the people of the Western world. In our own academic circle only one group rejects it, for its simplicity, the philosophers.

The solution of our problem is that, in its supernatural aspect, religion has been the most effective social cement, and thus, at least in considerable parts of history, the most potent factor in social evolution, and that in its moral aspect it has presented and enforced moral codes, very much the same which would have been recognized, but less perfectly observed, without its authority.

Since we interpret the past so confidently, we may be expected to forecast at least the immediate future, and to draw from the past some lessons for to-morrow's use. Here my confidence diminishes. The future begins with the present. There is no question that in our Western cultural world the development of science and the popular assimilation of some of its findings have impaired the hold of religion. And, quoting Spencer: "Few things can happen more disastrous than the decay and death of a regulative system no longer fit, before another and fitter regulative system has grown up to replace it."

Hardly any thinking man will deny that this is the condition to-day. You have only to consider the public attitude toward industry and thrift to realize that the virtues which preserved our grandparents are flouted and scorned. Without the saving virtues, whereby do we hope to survive? Or is humanity going to the dogs?

Well, there are many who hope for "moral rearmament": that we may retrace a little and take up the old way to a better future. Such things have happened. Men foolish enough to be proud of their emancipation from religion have been glad to get back to it under the

scourge of misery. Sixteen hundred years ago, Christianity was welcomed by a very sick, free-thinking world. The Dark Ages followed, but it might have been worse. It might happen again; but we are not yet ready.

Let me quote Spencer again: "Now that moral injunctions are losing the authority given by their supposed sacred origin, the secularization of morals is becoming imperative." The secularization of morals may mean either of two things, or both. One of these is the formulation of an ethic code based on the demonstrable survival value of its articles, and the promulgation of this code by education. Such a code can be apodictic in its essentials, and therefore effective. It puts foolishness in the place of sin in religious codes, and of crime in the law of the state. This sounds weak; but the fact is that, while a man may reject the idea of sin, and take his chance with the law, no man willingly makes himself a fool in his own esteem. The difficulty with this solution of our troubles is not in making the code, nor in proving its soundness, but in teaching it. This solution might or could work; but I am afraid that it will not.

The other method of secularizing morals is by the assumption of complete control by the state. Every state practices this to some extent when it defines a wrong act as a crime. The extension of this practice is contrary to the American idea of personal freedom. The theory of the totalitarian state demands its extension; and some such states control the conduct of the individual in great detail. However repugnant we find this practice, I believe we must grant that a people who break away from the teachings of religion, and permit their morality to become as lax as ours has become, is at a serious, and eventually at a fatal disadvantage in competition with any people at all equal in other respects, which effectively maintains its code of conduct, by whatever means.

Humanity is not going to the dogs. But our particular race is as capable as any other of damning itself by its own foolishness.

OBSERVATIONS ON THE ECOLOGY AND NATURAL HISTORY OF ANURA

I. HABITS, HABITAT AND BREEDING OF *BUFO COGNATUS* SAY¹

ARTHUR N. BRAGG

(Continued from page 349, July-August, 1940, issue)

ECOLOGICAL RELATIONS OF THE TADPOLES

The pools in which *Bufo cognatus* breeds are very productive of foods in the limnological sense. The detail of the ecological seasonal succession in them has never been worked out carefully, but, in general terms, it is as follows (see also Barkley and Smith, 1934):

Within a few days after rains fill the buffalo wallows (more or less, depending upon prevailing temperatures), single-celled algae make their appearance and thousands of eggs of small Crustacea hatch. Protozoa and turbellarian worms may be found in large numbers on the jellies of the toads' eggs within twenty-four hours of their production. By the time that the tadpoles hatch or very shortly thereafter, filamentous algae are usually abundant and aquatic insects (principally beetles of the families *Hydrophilidae* and *Dytiscidae* and various bugs) inhabit the pool. Water ferns (*Marsilea* or *Isoetes*) are often common. Tadpoles of other species of *Amphibia* (at least two species of *Pseudacris*, *Scaphiopus hammondi*, and, if the temperature is not too low, *Gastrophryne olivacea*) may also occur. Flowering plants begin to thrive about the edges of the pool in response to available moisture and these are visited by numerous small insects, some of which often fall into the pool. Feeding upon these insects, small frogs (especially *Pseudacris* spp. and *Acris gryllus*) usually occur, sometimes sparingly but often in abundance. Within a week or two, each pool is a veritable microcosmos, teeming with life ranging in type from bacteria and algae

¹ Contributions from the Zoological Laboratory of the University of Oklahoma, No. 197.

to insects and vertebrates, all in very complex interrelationships with the others. Often, conditions within a pool are kept somewhat in flux by cattle and horses; sometimes a pool which is beautifully clear when the toads breed becomes and remains quite roiled by the stomping of the larger animals. In such cases, the tadpoles seem to get along well, for I have followed hundreds of them through all stages in such pools and have seen them metamorphose.

The toad tadpoles fit into this picture in two different ways: (1) They feed upon algae, remains of insects and any other animal or vegetable matter which becomes sufficiently softened by the action of the water or bacteria. They are, therefore, partially scavengers. In laboratory cultures, they have been seen to feed upon algae, dead flies, flake fish-food, tropical-fish food, cooked rolled oats, dead tadpoles of their own species and, more rarely, calf's liver. (2) They serve as food for predatory insects and tadpoles, as discussed earlier.

In both capacities, they are of some significance to the succession of life in the pool because of their numbers. A single example is enough to bring this out.

The larger of the two buffalo wallows whose measurements were given above was observed carefully almost daily from early February to late May, 1938. Its level fluctuated from approximately seven to twelve inches, but it was never dry. If we assume it to be circular and to have averaged nine inches in depth, then its area was approximately 386 square feet and its volume, 290 cubic feet. *Bufo cognatus* produced two clutches of eggs in this pool on the same night and no others subsequently. Since the number of eggs in a clutch is approximately 20,000 (Bragg, 1937) and since almost all of these eggs hatched, there was an average of over 100 tadpoles to the cubic foot of water in this pool. Similar calculations on another pool gave an average of 770 tadpoles to the cubic foot.

Under these conditions, the struggle for survival must be great and often tends to become more intense as the pool shrinks from evaporation and the tadpoles become larger.

This tendency is lessened, however, by the depredations of predators. That an ecological balance is finally struck between the factors making for survival and those tending toward destruction of the tadpoles is shown by two facts: (1) that numerous larvae succeeded, both here and in comparable situations elsewhere, in passing metamorphosis and (2) the larvae emerged from another pool of a different type after a larval period of almost exactly the same duration as that of the larvae in this particular pool. This shows that the average of the ecological factors in the two places were closely comparable in so far as the toads were affected. *Scaphiopus* was not nearly so abundant in the second pool, but predaceous beetle larvae were much more numerous.

It is possible that these facts may be correlated with the observation that the tadpoles always remain small. At hatching, the larvae average about 3.0 mm in length, varying approximately between 1.7 and 3.5 mm. Just before metamorphosis, the average head-body length of twenty-two individuals was 11.2 mm, with an average weight (after preservation in 70 per cent. alcohol) of but 0.2 gm. The body as such actually weighed less than this because of the presence of the tail, which exceeds the head and body in length. It should be noted, however, that if there is such a correlation as that suggested, it applies equally well to most other bufonid tadpoles, at least of American species, since all these also always remain small. Even if the consistently small size of toad tadpoles be solely due to genetic factors, this size may well be an evolutionary adaptation to the crowded conditions resulting from the production of large numbers of eggs in relatively small amounts of water. Only further observation can determine with certainty whether this is the case.

METAMORPHOSIS

The length of larval life of *Bufo cognatus* is close to one and one-half months, although unfavorable conditions may lengthen it somewhat, at least in the laboratory (Bragg,

1937a). The following summary of field observations made in the spring of 1938 illustrate this:

Station 1 (buffalo wallows)

March 29	eggs laid.
April 29	many tadpoles of various sizes.
May 14	no tadpoles; thought to have completed metamorphosis and left the pools.
Total time		46 days.

Station 2 (buffalo wallows)

March 29		eggs laid.
March 31	.	late gastrulation.
April 18	tadpoles very numerous.
May 13	.	start of metamorphosis; hind-legs well developed, dorsal spots becoming evident, no forelegs.
May 14	.	Some with forelegs and long tails on or near the bank.
May 15	.	Many with but stumps of tails; a few as far as two feet from the pool.
May 16		Beginning to range away from the pool; most now showing the red peppering characteristic of the young toads.
May 18		The largest individuals are now from 50 to 60 feet from the pool. These no longer seek to enter the water for protection.
May 20		All that are near the pool are small; larger individuals have left the region of the pool permanently.
May 26	.	Older individuals found as much as 100 feet from the pool; some are still just emerging.

Total times:

Laying to start of metamorphosis	45 days.
Time consumed in metamorphosis	2 days. ²
Time that young toads remained close to pool	2 days.

At various times during the period from late March to mid-May, collections of eggs, embryos or tadpoles were made and reared in the laboratory for comparison with the developing young in the pools. In every case, some of the laboratory animals metamorphosed within a few days of the time that their sisters in the field transformed. However, whereas in the field nearly all which did not fall prey to predators lived and metamorphosed at about the same

² For about one week prior to this time, the hind legs grew rapidly. Therefore, I mean to indicate that the actual transformation from aquatic to terrestrial existence takes about two days. The whole process of metamorphosis is carried through in about one week.

time, those in the laboratory varied much in rate of development and many in each culture died and were eaten by those that remained. This occurred whether the water was changed frequently or not and whether tap-water or spring-water was used in preparing the cultures. As might be expected from the findings of Adolph (1931) and of Rugh (1934), tadpoles in uncrowded cultures developed better, on the whole, than those in crowded cultures.

The exact cause of these differences in laboratory-grown tadpoles and those from the same clutches which remained in the pools was not investigated. However, two considerations lead me to suspect a dietary factor. (1) All environmental conditions, in all probability, varied more in the field than in the laboratory. (2) In all cultures, some tadpoles early forged ahead of their fellows; one by one the others died, and when they were eaten by the remainder, some of these in turn forged ahead. Finally, out of several hundred, usually not more than a dozen or so eventually transformed; but those which were successful transformed at the same time as their sisters in the field and gave every evidence of being as healthy and vigorous. This suggests that some substance necessary to normal growth and development was available in limited quantities only and that it finally became concentrated in the bodies of the few that went through metamorphosis. Since the greatest difference in diet was a relative scarcity of animal matter in the laboratory cultures, the source of such a substance, if indeed one exist, is probably to be found in animal-food.

Size-variation at metamorphosis is not marked (see Table 1). The head-body length ranges from about eleven to about thirteen millimeters, and the tail is somewhat longer than this. Seldom does a tadpole reach a total length greater than thirty millimeters before transformation begins.

Metamorphosis is quite rapid (see Fig. 7). During the first day, the hind legs which have been present as visible rudiments for some time (since 20-25 mm total length,

TABLE 1
AVERAGE LENGTHS AND WEIGHTS (MM AND GR, TO THE NEAREST .01) OF
VARIOUS STAGES OF YOUNG *BUFO COGNATUS**

Stage	No. of indiv.	Length			Weight
		Head-body	Tail	Total	
A	22	11.20 +	14.94	26.24	0.20
B	35	12.39 -	16.25	28.64	0.20
		(a)	(a)	(a)	
C	7	12.33	14.93	27.26	
C	47	13.12	14.00	27.12	
C	12	15.13 -	14.00	29.13	0.18
D	102	11.29	0.32	11.61	0.15 -
E	158	12.03 -	(trace)		0.15 +
		(a)		(a)	
F	18	18.47		18.47	
		17.94		17.94	0.56 -
		(a)		(a)	(a)
G (sta. 2) .	12	56.26		56.26	21.77 -
		49.86		49.86	14.77
		(a)		(a)	(a)
G (sta. 4) .	27	44.91		44.91	12.15 +
		43.14		43.14	9.37
		(a)		(a)	(a)
G (total) ...	39	48.49		48.49	15.19 -
		45.22		45.22	11.03

* Those measured while alive are marked (a); others were measured after preservation in 70 per cent alcohol. The stages given are defined as follows: A, ready for metamorphosis, hind legs just beginning to enlarge but no forelegs present; B, start of metamorphosis, hind legs considerably enlarged; C, Two pairs of legs present, tail still long, seeking to leave the water; D, tail but a stub, out of water but very close to edge; E, end of metamorphosis, dispersing in all directions from the pool; F, about one week after dispersal from the pool; G, end of first summer, about four months after metamorphosis.



FIG. 7. Stages at and just after metamorphosis of *Bufo cognatus*. The two largest specimens have been out of water approximately one week. Note the evidence of very rapid growth.

Bragg, 1936a) enlarge noticeably, the anterior region of the body and head enlarges and appears to become depressed, and narrow brownish black bars become evident on the dorsal surface of the legs. There is still no noticeable reduction in the length of the tail, which is still used actively in swimming (Table 1, stages B and C). By the end of the first day of active metamorphosis, the animals tend to congregate in large numbers near the bank, become less active unless disturbed, and apparently take little if any food. Within a few hours, the forelimbs break through, sometimes the one on the right preceding that on the left and sometimes, *vice versa*, the hind legs grow rapidly, and there is some decrease in weight (Table 1, stage C).

By the middle of the second day, the color pattern has changed radically. On the dorsal surface, large, dark-colored spots appear. These are usually arranged approximately in pairs, four to eight in number, but with five or six the more common condition. These are the forerunners of the large dorsal spots characteristic of the adults and constitute one of the principal features by which very young toads of this species may be distinguished from toads of other species at the same stage. Often members of certain of these pairs of spots run together in the midline and sometimes one of a pair exceeds its fellow in size. A little later, similar spots, smaller in size, appear along the lateral surfaces, especially in the region of the head. The lateral spots often occur close to the same anterior-posterior level as the dorsal spots, but they vary more than the latter in exact distribution. The forelimbs soon become barred like the hind legs. The ventral iridescence of the tadpoles, described earlier (Bragg, 1936a), has faded by this time, and the under-surface now presents a uniform, just-off-white color. The general tone between the characteristic dorsal and lateral markings is brown or grayish-brown, and this changes abruptly in the ventrolateral regions to the lighter color of the ventral surface. At a magnification of forty, the skin on all surfaces appears pebbly due to tiny wart-like protuberances.

While these changes are occurring, the structure of the head has altered. Larval teeth have disappeared, the mouth has widened, and the eyes have become somewhat more prominent. Also, the behavior of the tiny toads has changed; each now sits with its head out of water at the very edge of the pool and moves very little except when disturbed. The tail is still long and functional, as one easily observes upon approaching a pool ringed with these little toads. At such times, each takes to the water and swims actively away.

Within another few hours, the toads come completely from the water. The tail now begins a very rapid degeneration so that by the end of the second day or early in the third only a small stump remains. Each toad now sits at the very edge, facing the pool into which it will hop if disturbed. Now, however, the hind-legs are functional and the little toad does not go beneath the water; instead, it swims feebly at the surface, usually not getting the dorsal portion of its body wet.

At about this time, a new feature of the coloration develops. This takes the form of brick-red speckles, more or less uniformly distributed over the dorsal and lateral regions. These speckles are quite numerous. On the large dorsal spots, this reddish peppering contrasts sharply with the darker background.

The above description is based upon what is considered to be the average condition. Actually, there is considerable variation among individuals. For example, some of them develop the dorsal speckling before the tail has decreased much in length; in others, it does not appear till the tail is nearly gone. In a few individuals, the mouth may be well transformed, including the development of a prominent tongue, before the tail is appreciably absorbed, though this is not usual. In general it seems that, with the great transformation taking place so rapidly in all portions of the body, the synchrony of the various processes involved in it is not near enough to perfect for all individuals to show exactly the same conditions at any one stage. Transformation in nature is very successful, for

of the several thousands which I have watched passing through the process I know of none which died from physiological causes.

The recently metamorphosed toads start feeding immediately. The food consists of various types of animals which are small enough to swallow. Examination of fecal masses has revealed the following: mites, in considerable numbers; various small beetles, including several small ground-beetles (*Carabidae*); young snails (*Physa*); and evidences of several other types of small insects, the remains of which were not recognizable. In the laboratory, toads two days after metamorphosis took gnats, spiders, leaf-hoppers and small beetles. They did not seem interested in any insects as large as or larger than house-flies.

Evidently, feeding sometimes starts before the digestive system is fully prepared, for one fecal mass contained a live mite which had passed through the digestive tract of a toad apparently unharmed.

As might be expected, the size of the body as measured by either weight or length decreases during metamorphosis (Table 1). With the beginning of effective feeding, however, there is a very rapid growth. Within a week after the toads have left the vicinity of the pool after metamorphosis, some individuals have increased over 200 per cent. in weight, although many are much smaller than this. The toads are still tiny, however, the largest weighing less than a gram (after preservation in 70 per cent. alcohol). The animals are much more variable in size now; and this variation continues throughout their first summer if one can judge by the several sizes of young toads collected in the fall.

On October 10, 1938, after a light shower in the morning, young toads were out in numbers in the evening. A total of thirty-nine was collected, twelve within one mile of a known breeding site (station 2) and twenty-seven within a half mile of another (station 4). Since breeding during 1936 and 1937 was not successful, it is certain that these individuals were produced in the spring of 1938; they were, therefore, about four months of age. As measured while

still alive, those from near station 2 varied from 12.0 to 37.2 g. in weight (average, 21.77 g.) and from 48.0 to 63.0 mm in length (average, 56.26 mm). From the other locality the individuals were smaller—weight, 7.7 to 20.3 g. (average, 12.15 g.); length, 36.0 to 56.0 mm (average, 41.91 mm). If we compare the average length of all these (48.49 mm) with the length of adults given earlier, it is at once apparent that these toads were approximately half grown. (Compare, also, Figs. 3 and 8.) If during the second sum-



FIG. 8. *Bufo cognatus* approximately four months of age. The specimens shown are the smallest, the largest and one of intermediate size of those taken on October 10, 1938. The largest specimen is more than half the size usually attained at maturity.

mer the same rate of growth should be maintained, then some individuals, at least, might reach the adult size during the second year. Since there was a great variation in sizes, however, it seems probable that some individuals would not reach maturity for at least another year.

Smith (1934) quotes Dickerson as follows: "Judging from the series of decidedly different sizes of this toad (i.e., *B. cognatus*) found in the spring, at least five years must be required for full growth to be attained." My

observations indicate that the different sizes mentioned may not be the result of differences in age alone but also of the availability of food to individuals. In relatively moist summers (like that of 1938) more nights will be spent in hunting for food, and growth will be correspondingly more rapid. During dry summers, however, the young toads remain hidden in burrows, as do the adults, even during the night; and relatively little food is taken. The age at maturity, therefore, varies with individuals and is mostly dependent upon available food.

It is of some interest that of the thirty-nine young *B. cognatus* collected during the evening of October 10, 1938, all but two were females. Of twenty-one young *B. w. woodhousii* collected at the same time, twenty were females. It is also of interest that no adults of *B. cognatus* and only one of *B. w. woodhousii* were seen, although the young of both species were very abundant, much more so, in fact, than the number collected might seem to indicate.

These facts suggest one of two possibilities: (1) the young female toads are more responsive to the presence of a small amount of moisture than are the males; or (2) there were many more females produced during the previous breeding season than males. Both seem equally possible and I have had no opportunity to check which is correct, since this one evening was the only time that *B. cognatus* has appeared during the fall, due to exceptionally dry weather. In any case, it appears likely that the young females are more responsive to the presence of slight moisture than are the adults of either sex.

SUMMARY

Bufo cognatus Say may be found in grassland areas of the Great Plains from southwestern Minnesota southward into Mexico and southwestward to the Imperial Valley, southern California. Its habitat varies in different localities; it is present only in lower, moister areas in arid or semi-arid regions (Texas, New Mexico, Arizona) but in central Oklahoma it occupies the higher portions of the plains. It is largely nocturnal in habit but often appears

during daylight in moist weather, especially during the breeding season. Dry conditions often keep these toads in underground burrows for weeks at a time.

Nothing is known with certainty about the natural enemies of adults. They are probably eaten sparingly by snakes and hawks. Thousands are killed each year by automobiles.

They breed only after rain in spring or summer when the temperature exceeds 12° C. Breeding sites in central Oklahoma include buffalo wallows, flooded fields and the edges of extensive temporary pools. They do not use ditches, "tanks" in pastures, streams nor lakes, and they have never been known to breed in excessively muddy water. Differences in behavior of the sexes during breeding insure that most clutches of eggs will be laid in the location most favorable for development through metamorphosis. The eggs are very numerous (20,000 to each full clutch), and the percentage of hatching is high. Concentration of tadpoles in some pools may exceed 750 per cubic foot of water. Food for the larvae (algae and plant and animal debris) is plentiful in the pools, and large numbers of young often succeed in passing metamorphosis. Their number is limited by the following factors: (1) all pools may dry up and the tadpoles in them die; (2) certain pools may become contaminated and thus kill all eggs and larvae in them; (3) predators often take a heavy toll (predaceous tadpoles of *Scaphiopus* and larvae of water beetles are known to eat the tadpoles); and (4) at and slightly after metamorphosis, birds (crows and possibly others) sometimes eat the young. The embryos and tadpoles can withstand temperatures in the pools of from near freezing to at least 37° C. and probably more.

Metamorphosis starts about one and one-half months after eggs are laid at a tadpole length of 26 to 29 mm. The young toads have a distinctive color pattern, the major feature of which is four to eight pairs of large dark spots on the dorsal surface and smaller spots on the sides. In two-day-old toads, the whole dorsal surface is dotted with small, brick-red speckles.

Immediately after transformation, the young toads average about 11.0 mm in length, and there is not much variation among them. When at two to three days of age, they disperse from the pool, they are about a millimeter longer. They immediately start feeding and grow very rapidly upon a diet of small insects, young snails and mites. In one week they vary much in size, the larger reaching as much as 20.0 mm in length. In about four months some may be half grown (over 50.0 mm in length), but others may remain much smaller. It is probable that the rate of growth of an individual is primarily dependent upon availability of food. If this be the case, then adult size may be reached in two years by some individuals but not attained by others for three or four years. In any case, difference in size among these young toads is not always indicative of difference in age, as assumed by Dickerson (1913).

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EVOLUTIONARY ALLOMETRY IN THE SKELETON OF THE DOMESTICATED DOG

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INTRODUCTION

It is well known that within a group of related types of organisms the adult proportions are frequently correlated with total body size. Recent investigations have shown further that in a number of instances the relation between the sizes of corresponding parts in the adults of different types composing such a group conforms to the simple power function

$$y = bx^a$$

where x and y are the sizes of two parts and b and a are constants (Hersh, 1934; Robb, 1935a, 1936; Hamai, 1938).

This expression is identical in form with the law of allometry (Huxley and Teissier, 1936) which has been found in a large number of cases to be a valid empirical representation of ontogenetic growth relations in a single type (see Huxley, 1932). Hence the aforementioned group-phenomenon may be termed "evolutionary allometry."

The investigations cited above deal with size relations in groups of species or higher taxonomic categories. In view of the value of this approach in clarifying taxonomic and evolutionary relationships, it is also of interest to examine its applicability to genetic varieties within a species. For such a study the domesticated dog is exceptionally suitable. Not only does the species comprise a large number of breeds varying greatly in both adult body size and proportions, but it also offers the possibility of supplementing the group investigations with genetic analyses of ontogenetic relative growth in the individual breeds and their hybrids.

Skeletal proportions have commonly been employed as

a basis for the classification of dogs. The descriptive methods of earlier investigators provide adequate diagnostic features for distinguishing the individual types, yet they have proved inadequate as a basis for classifying these into larger subspecific categories, and efforts in this direction have led to considerable disagreement. The problem has recently been clarified to a large extent, however, by the thoroughgoing statistical analysis of Wagner (1930).

The procedure employed by Wagner consisted in plotting various size indices of different breeds against the length of the cranial cavity, which was selected as a measurement representative of body size. For some indices, the points for the various breeds formed a single band, but for the most part two distinct bands were obtained, the breeds occupying each being the same in all cases. From these results Wagner concluded that the species *Canis familiaris* may be subdivided into two major groups, which he designates simply as Groups *a* and *b*.

The present investigation consists primarily of a further analysis of Wagner's data in terms of the law of allometry.

MATERIAL AND METHODS

The data taken from Wagner (1930) on which the present analysis is based include measurements on the skulls of 247 specimens of 28 modern breeds and of 17 European wolves (*Canis lupus*), also measurements on the limb bones of 146 specimens of 18 breeds. The breeds included, in the grouping proposed by Wagner, are as follows:

- | <i>Group a</i> | |
|-----------------------|----------------------|
| 1. German shepherd | 7. Poodle |
| 2. Dachshund | 8. Dunker |
| 3. Setter | 9. Lapland dog |
| 4. Dobermann pinscher | 10. Iceland sheepdog |
| 5. Pointer | 11. Fox terrier |
| 6. Dingo | 12. Schnauzer |
| | 13. Borzoi |

- | | |
|---------------------------------------|---------------------------------------|
| 14. English greyhound | 21. Saint Bernard |
| 15. Large whippet | 22. Newfoundland dog |
| 16. Whippet | 23. Bulldog |
| 17. Rat terrier | 24. Old-type bulldog |
| 18. Bolognese | 25. French bulldog |
| 19. Toy terrier and monkey
terrier | 26. Boxer |
| Group b | 27. Pug |
| 20. Great Dane | 28. Pekingese and Japanese
spaniel |

It may be noted that in some cases similar breeds have been lumped together; for example, no distinction is made between different breeds of setters or pointers, and such forms as the pekingese and Japanese spaniel are treated as one group. On the other hand, two types are included which do not represent recognized breeds, namely, the large whippet and old-type bulldog. These include a number of specimens markedly larger than the usual whippet or bulldog, and hence separated by Wagner.

In the selection of measurements suitable for analysis, it was found that only those made between accurately determinable landmarks could be successfully used. In this respect most of the cranial measurements given by Wagner (*e.g.*, cranial width, cranial height) are particularly unsatisfactory, since the landmarks involved are only vaguely fixed and, consequently, these measurements are highly variable. For the precise landmarks and techniques employed in making the measurements used in the present investigation, the reader is referred to Wagner's paper.

Although the literature contains a considerable quantity of data collected by other investigators, it was for the most part impossible, because of differences in methods of measurement, to make use of them. Some measurements, however, such as zygomatic width and basilar length of the skull, were found to be fairly uniform in character, and in the analysis of the relationship between these two dimensions, measurements on thirty-one specimens of ancient and prehistoric types, taken

from a number of sources, were included. Also, some of the data of Becker (1923) on the German shepherd were found sufficiently comparable to those of Wagner to warrant their use in determining certain ontogenetic relative growth constants for this breed. The nature of the data used in these two instances will be discussed more fully below.

It can readily be seen that if the law of allometry holds, then a graph of $\log y$ against $\log x$ should yield a straight line with slope α and y -intercept $\log b$. The constant α is of particular interest since it represents the per cent. increase in y for each 1 per cent. increase in x . For each of the relationships investigated, therefore, the average measurements for each breed were plotted on a double logarithmic grid. Curves were

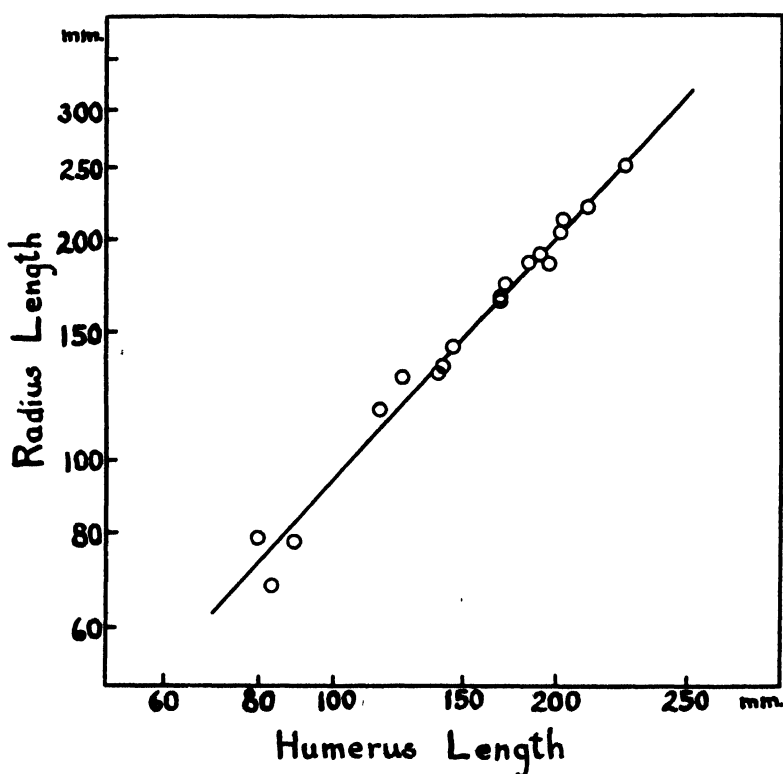


FIG. 1. Double logarithmic plot of radius length against humerus length.

fitted to the data by the regression method of least squares (Feldstein and Hersh, 1935).

RESULTS

The limb bone relations. Three relations involving the limb bones were investigated, namely radius length / humerus length, tibia length / femur length, and length of hindlimb (femur + tibia) / length of forelimb (hum-

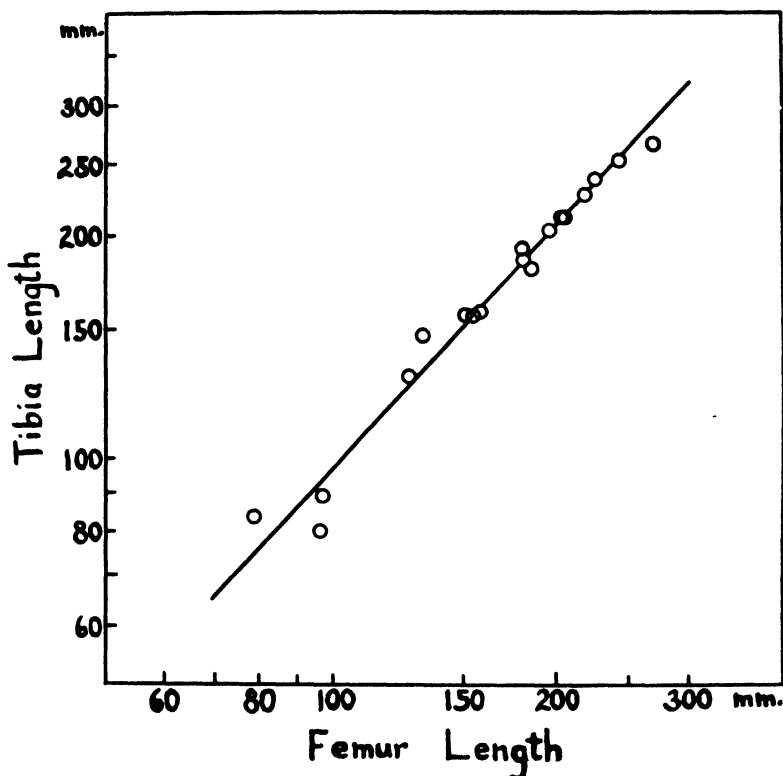


FIG. 2. Double logarithmic plot of tibia length against femur length.

erus + radius). The measurements selected as best representing the lengths of the humerus, radius and tibia are those designated by Wagner as *grösste Länge*, while that used to represent femur length is Wagner's *grösste Trochanterenlänge*. These particular measurements were chosen chiefly because they conform to those

employed by a number of anthropologists in the measurement of primate limb bones.

The curves obtained are shown in Figs. 1-3. It will be noted that in all three cases the points, with few exceptions, fall closely along a single straight line. The

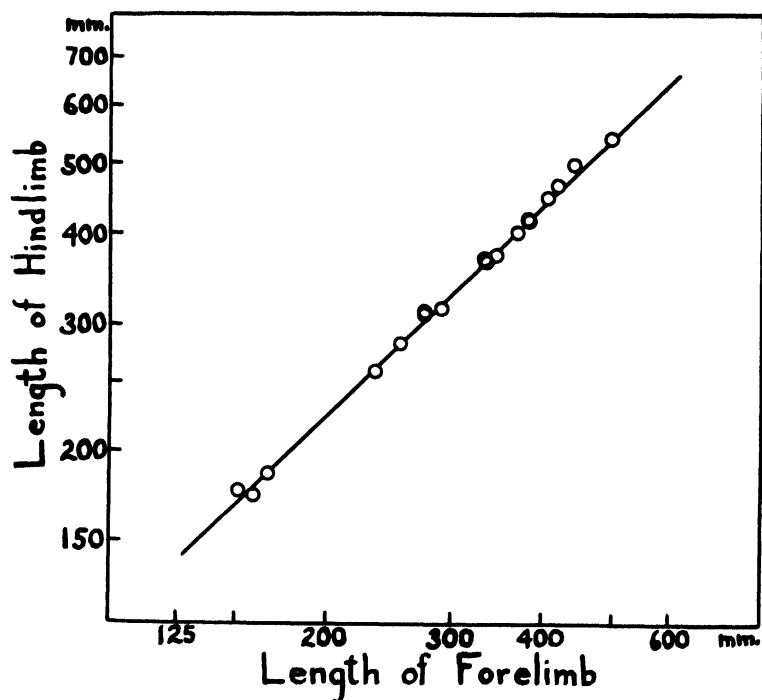


FIG. 3. Double logarithmic plot of length of hindlimb against length of forelimb.

points showing the greatest deviation in Figs. 1 and 2 are those representing the dachshund. In view of the pronounced achondroplasia occurring in the limbs of this breed, these deviations are perhaps not surprising.

The values of α , given in Table I, are somewhat greater than 1 for the first two relations, and slightly less than 1 for the third. These results indicate that among the breeds investigated the lower limb segments tend to become relatively somewhat longer with increasing limb length. On the other hand, there is comparatively little

change in the relative lengths of the limbs, the hindlimb in all breeds being somewhat longer than the forelimb.

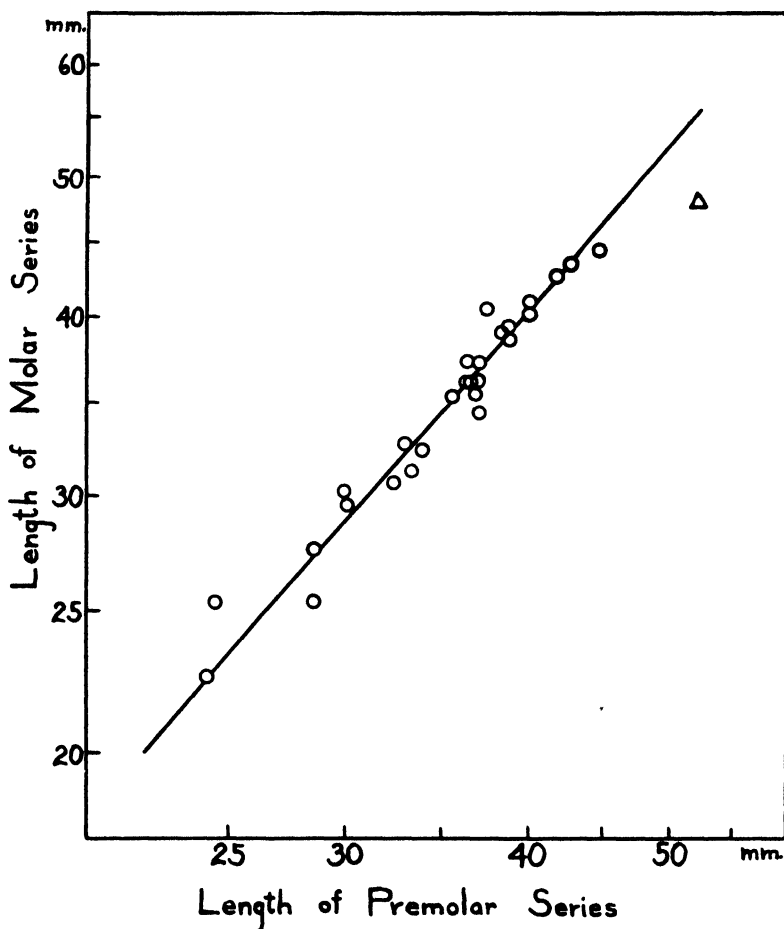


FIG. 4. Double logarithmic plot of length of molar series against length of premolar series in the lower jaw. Triangle represents the European wolf.

The molar / premolar relation. Fig. 4 shows the curve obtained by plotting logarithmically the length of the molar series of the lower jaw against the length of the premolar series. Here again all the breeds concerned occupy a single curve, although the points exhibit an appreciably greater scatter than in the case of the limb relations. The value of α (Table I) is greater than

unity, indicating that with increasing length of the lower jaw, the length of the molar series relative to that of the premolar tends in general to increase.

With respect to this relation, the wolf deviates markedly from the curve characterizing the various breeds of dogs. Not only are the two series of teeth in the wolf greater in absolute length than in any of the dogs, but there is also a difference in proportions, the wolf having a relatively shorter molar series than any type of dog. These differences have also been noted by Wagner (1930).

TABLE I

VALUES OF b AND a FOR THE LIMB BONE, MOLAR/PREMOLAR AND PALATE LENGTH/
BASILAR LENGTH RELATIONS

Relation	b	a
Radius length/Humerus length	0.597	1.098
Tibia length/Femur length	0.642	1.090
Hindlimb length/Forelimb length	1.294	0.972
Molar series/Premolar series	0.558	1.161
Palate length/Basilar length	0.506	1.015

The palate length / basilar length relation. In this case, as in the preceding ones, the points for all the breeds form a single curve. As may be seen from Table I, a is approximately equal to 1, and b is about 0.5. That is to say, the palate length is about one half the basilar length in all the breeds. In this respect, the wolf does not deviate from the dogs.

The snout length / cranial length relation. In Fig. 5, the logarithm of the snout length is plotted against the logarithm of the length of the cranial cavity. In this and in the remaining figures, the breeds falling in Wagner's Group *a* are denoted by open circles and those in Group *b* by crosses.

It may be noted that the two groups are characterized by distinct curves. The breeds of Group *b* all occupy one curve, while those of Group *a* form two separate curves, the upper including breeds 1-16 and the lower breeds 17-19 of the list given above (p. 440). Curves 1 and 2 of the figure approach one another at their upper ends and would intersect, if continued, at about the point representing the wolf.

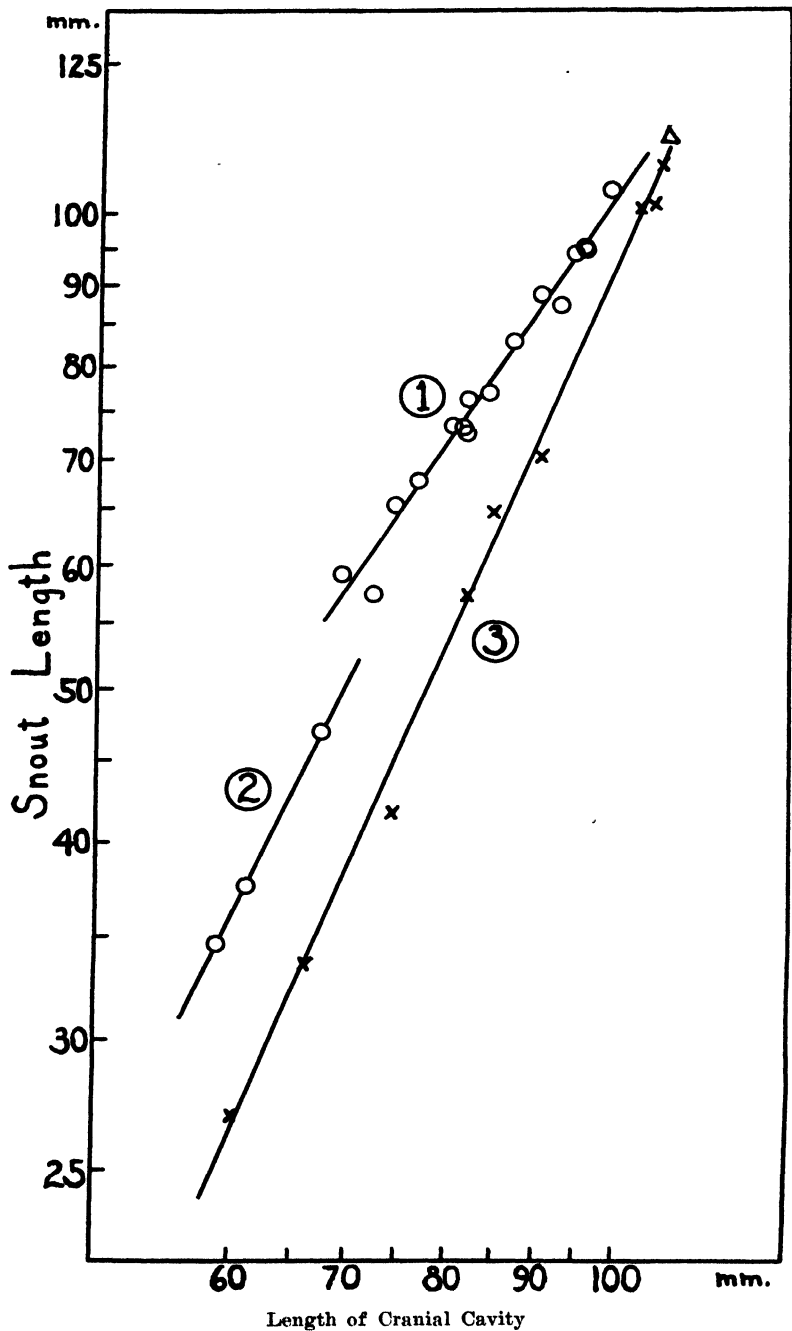


FIG. 5. Double logarithmic plot of snout length against length of cranial cavity. Circles represent breeds in Group a, crosses those in Group b, and triangle the European wolf.

The values of α , which are given in Table II, are all high, indicating a sharp increase in the relative length of the facial portion of the skull with increasing cranial length. This trend is one which has been previously noted by numerous investigators, and which is known to be paralleled by a similar ontogenetic trend in individual breeds (see for example Becker, 1923; Weidenreich, 1925; Sommer, 1931).

TABLE II

VALUES OF b AND α FOR THE SNOUT LENGTH/CRANIAL LENGTH (SL/CL), SNOUT LENGTH/JAW LENGTH (SL/JL), PALATE WIDTH/PALATE LENGTH (PW/PL), OCCIPITAL WIDTH/BASILAR LENGTH (OW/BL), AND ZYGOMATIC WIDTH/BASILAR LENGTH (ZW/BL) RELATIONS

Relation	I		II		III	
	b	α	b	α	b	α
SL/CL	0.058	1.624 (1)	0.004	2.249 (2)	0.0009	2.505 (3)
SL/JL	0.347	1.106 (1)	0.204	1.204 (2)	0.0004	2.581 (3)
PW/PL	2.764	0.694 (1) (1)	5.160	0.596 (3)
OW/BL	1.181	0.781 (1) (1)	10.893	0.313 (3)
ZW/BL ...	3.345	0.670 (1) (1)	8.434	0.504 (3)

Relation	IV		V		VI	
	b	α	b	α	b	α
SL/CL (3) (3)	0.058	1.624 (1)
SL/JL	0.013	1.716 (4)	2.878	0.687 (5)	0.347	1.106 (1)
PW/PL	20.011	0.242 (4)	0.393	1.119 (5)	3.659	0.604 (2)
OW/BL ...	4.171	0.560 (4)	0.751	0.877 (5)	2.208	0.640 (2)
ZW/BL ...	20.768	0.351 (4)	0.760	0.961 (5)	5.460	0.553 (2)

Note: The Roman numerals at the heads of the vertical columns refer to the groups listed on p. 455. The numbers in parentheses indicate the number of the curve in the appropriate figure to which the constants apply.

The snout length / lower jaw length relation. The curves obtained for this relation are shown in Fig. 6. The breeds of Group *a* show the same subdivision as in the preceding relation; those of Group *b*, on the other hand, form three distinct curves. Curve 3 includes breeds 27 and 28; curve 4, breeds 23-26; and curve 5, breeds 20-22.

All the curves, with the exception of curve 5, have a slope greater than 1 (see Table II). Curves 3 and 4 have especially high values of α , which means that in these groups the snout length tends to increase considerably in relation to the length of the lower jaw with increasing skull size. In this connection, it may be

pointed out that in the larger breeds of bulldogs the lower jaw is generally less undershot than in the smaller breeds. Thus Wagner (1930) remarks that in the old-

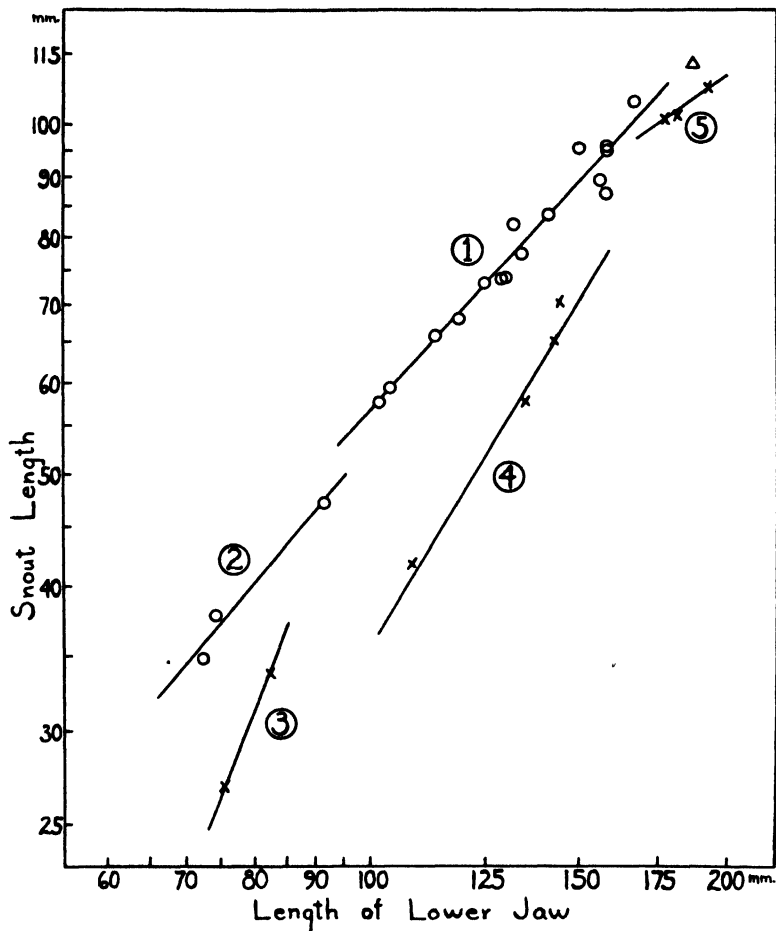


FIG. 6. Double logarithmic plot of snout length against length of lower jaw. Circles represent breeds in Group *a*, crosses those in Group *b*, and triangle the European wolf.

type bulldog, the largest breed of the group, the jaw is either only slightly or not at all undershot.

It should be noted that the point representing the wolf does not in this case fall at the intersection of two curves, but instead appears to conform rather closely to curve 1.

The width / length relations of the skull. Three relationships of this type were investigated, namely palate width / palate length, width of occipital triangle / basilar length, and zygomatic width / basilar length. The resultant curves are shown in Figs. 7, 8 and 9.

It is evident from these figures that the curves obtained are essentially the same in all three cases. Group *b* in each case yields three curves, each of which includes the same breeds as the corresponding curve for the snout length / length of lower jaw relation. Group *a* splits into two curves, of which curve 1 includes breeds 1-12 and 17-19, and curve 2 breeds 13-16. This grouping, it should be noted, does not correspond to the one obtained for Group *a* in the preceding relationships.

In each of the three figures, all the curves except curve 5 have values of α considerably less than 1 (see Table

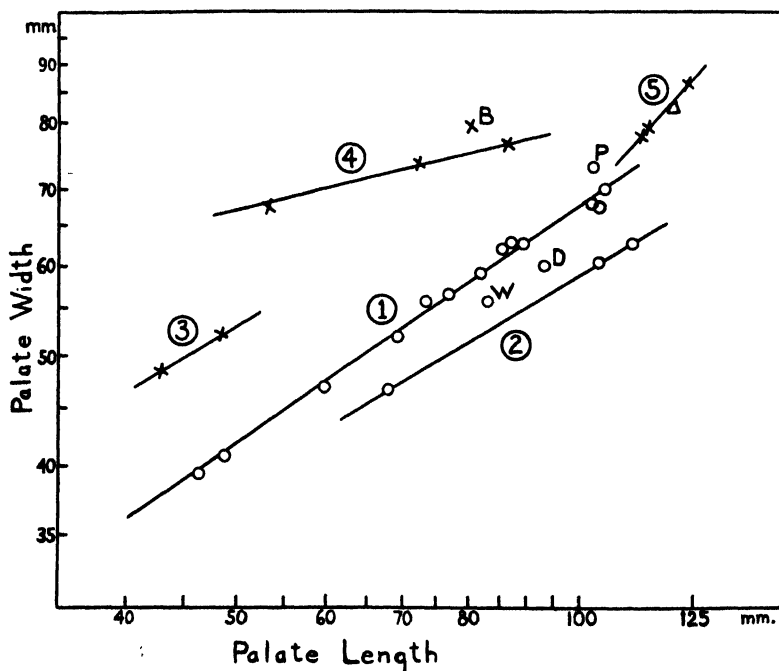


FIG. 7. Double logarithmic plot of palate width against palate length. Circles represent breeds in Group *a*, crosses those in Group *b*, and triangle the European wolf. B—boxer, P—pointer, W—large whippet, D—dingo.

II). There is thus for the most part a very marked decrease in skull width relative to skull length with increasing size, a decrease which is most pronounced in the bulldog group (curve 4). Here again it may be pointed out that a similar change in proportions has been found by numerous investigators to occur in the ontogenetic development of many breeds.

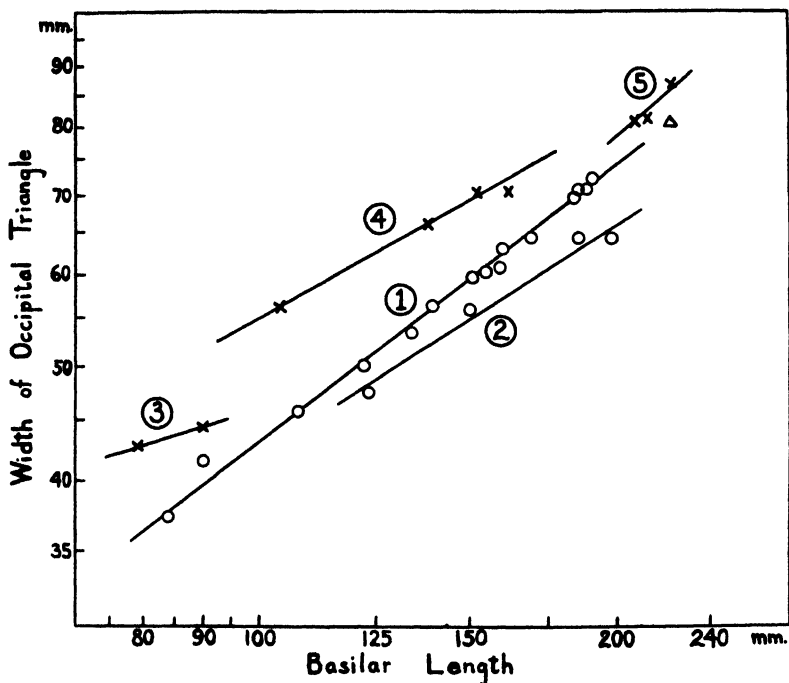


FIG. 8. Double logarithmic plot of width of occipital triangle against basilar length. Circles represent breeds in Group *a*, crosses those in Group *b*, and triangle the European wolf.

In the analysis of the zygomatic width / basilar length relation, measurements on a number of prehistoric and ancient specimens were included. These measurements were taken from the papers of Brinkmann (1924), Hasebe (1924), Hiltzheimer (1913, 1932) Noack (1916), Studer (1905), Teodoreanu (1926); and Wagner (1930). In a total of thirty-one specimens, nine distinct varieties are represented. These, together with the number of

specimens of each, are as follows: *C. f. palustris* (10); *C. f. palustris ladogensis* (6); *C. f. matris optima* (6); *C. f. inostranzewi* (5); *C. f. inostranzewi* (bulldog-like variety) (5); *C. f. intermedius* (3); *C. f. poutiatini* (1); primitive greyhound (1), Inca bulldog (1).

The mean values for each of these varieties are plotted in Fig. 9, where they are denoted by circles marked with crosses. It may be observed that six of the nine points

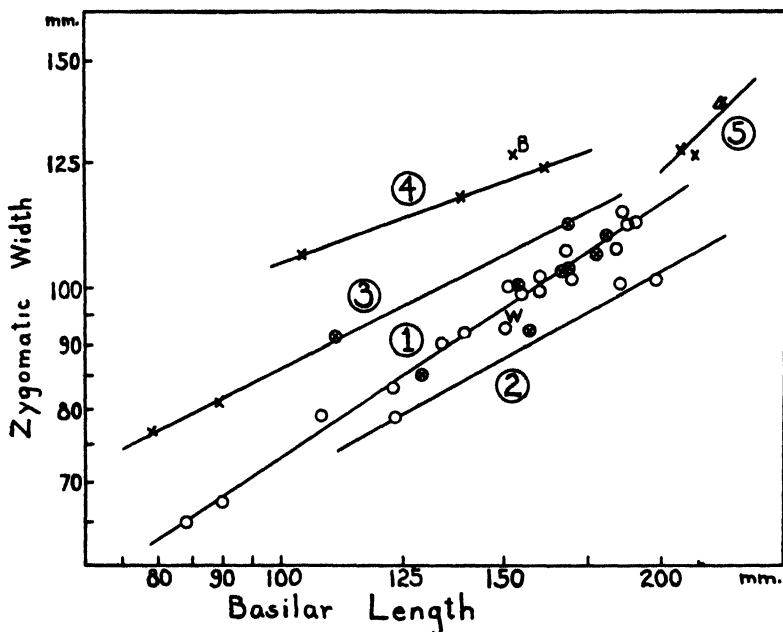


FIG. 9. Double logarithmic plot of zygomatic width against basilar length. Circles represent breeds in Group *a*, crosses those in Group *b*, crossed circles prehistoric and ancient types, and triangle the European wolf. B—boxer, W—large whippet.

conform rather closely to curve 1. Of the remaining three, that representing the primitive greyhound lies close to curve 2, while those representing the Inca bulldog and the bulldog-like variety of *C. f. inostranzewi* fall on curve 3. Hence, so far as is indicated by the available data, the relationships established for the modern breeds appear to hold also for these prehistoric and ancient types.

It is significant that the two bulldog-like forms do not occupy the same curve as the modern bulldog, but fall, together with the pug and the pekingese, on a curve intermediate between curves 1 and 4. Students of the history of the dog are generally agreed that the bulldog is derived from the same stock as the English mastiff, apparently by selection for smaller body size, the Bordeaux dog being regarded by some as an intermediate stage in this process (see Jesse, 1866, Vol. 2, p. 390; also Pötting, 1909). Both these breeds, therefore, should be expected to fall on the same curve as the modern bulldogs. The present results indicate in addition that the prehistoric and ancient bulldog-like forms are most probably not to be regarded as direct ancestors of the modern bulldog, but rather, as Wagner (1930) suggests, as somewhat similar forms which have arisen independently and from an entirely different stock. Similarly, the present results do not support the contention of Pötting (1909) and others that the pug represents a dwarf form of the bulldog.

In connection with these questions, the value of this analysis would clearly be enhanced by the inclusion not only of the mastiff and Bordeaux dog, but also of such dwarf forms as the griffons and Boston terrier. Unfortunately, no suitable data on any of these breeds are available.

There are several distinct deviations from the group curves which merit further consideration. These are, in Fig. 7, the points representing the pointer, large whippet, dingo, and boxer, and in Fig. 9, those representing the large whippet and boxer.

In the pointer, as Marchlewski (1930a) states, a broad facial portion with a marked indentation, or "stop," is regarded by fanciers as an essential feature. A not uncommon defect is "snipeness," in which the snout is narrow and pointed rather than broad and square. The pointer thus represents the result of selection of extreme variants with respect to snout shape, and it is this which

is apparently responsible for its exceptional position with reference to the palate width / palate length relation. Wagner (1930) finds that this breed is exceptional also in the degree of curvature of the face, which he attributes to selection for a pronounced "stop."

The large whippet is a form which occasionally crops up in whippet breeding. Since it is well known that the whippet is historically the product of a cross between the fox terrier and the English greyhound, the occurrence of these large forms is not surprising, nor is the fact that they are intermediate in some respects between the terrier and greyhound groups.

The dingo, or Australian wild dog, has been found by Longman (1928) to differ from other breeds of dogs in its dentition. In particular, the anterior-posterior length of the carnassial in the dingo is greater than 10 per cent. of the condylobasal length of the skull, whereas in all the types of domesticated dogs investigated it is less than 10 per cent. In this respect, according to Longman, the dingo resembles the wolf more closely than it does the domesticated dog. Wagner (1930) finds that the degree of curvature of the face is appreciably less in the dingo than in other dogs with the same skull size. The deviation in proportions of the upper jaw is very probably associated with these differences.

The German boxer, a dog with an extremely broad, short skull, appears to be very closely related to the bulldogs. The available information on this breed, however, does not provide any immediate explanation for its deviations from the bulldog group.

The point representing the wolf falls definitely on curve 5 in Fig. 7 and 9. In Fig. 8, on the other hand, it conforms much better to curve 1.

Allometric tribes. The results presented in the foregoing sections show that the domesticated dog may be subdivided into several groups, each of which is characterized by the fact that for any given relationship all its members fall along a single curve conforming to the

law of allometry. As a designation for any subspecific group distinguished in this manner, the term *allometric tribe* is proposed.

Group *a* comprises chiefly one large subdivision from which, in Figs. 5 and 6, the three smallest breeds, and, in Figs. 7, 8 and 9, the greyhounds and whippets are split off as separate groups. On this basis, Group *a* may be divided into three tribes, of which one is considerably larger than the other two. Group *b* likewise consists of three tribes, making a total of six. These tribes, each designated by the name of a typical form, are as follows:

<i>I Terrier Tribe</i>	Toy terrier and monkey terrier
German shepherd	<i>III Pug Tribe</i>
Dachshund	Pug
Setter	Pekingese and Japanese spaniel
Pointer	<i>C. f. inostranzewi</i>
Dobermann pinscher	(bulldog-like variety)
Dingo	Inca bulldog
Poodle	<i>IV Bulldog Tribe</i>
Dunker	Bulldog
Lapland dog	Old-type bulldog
Iceland sheepdog	French bulldog
Fox terrier	Boxer
Schnauzer	<i>V Great Dane Tribe</i>
<i>C. f. palustris</i>	Great Dane
<i>C. f. palustris ladogensis</i>	Saint Bernard
<i>C. f. matris optima</i>	Newfoundland dog
<i>C. f. inostranzewi</i>	<i>VI Greyhound Tribe</i>
<i>C. f. intermedius</i>	Borzoi
<i>C. f. poutiatini</i>	English greyhound
<i>II Toy Terrier Tribe</i>	Whippet
Rat terrier	Large whippet
Bolognese	Primitive greyhound

Each of the above tribes is characterized by a set of values of *b* and *a*, of which some are distinctive while others are the same as those of other tribes. The extent to which the values of *a* for a particular tribe differ from unity may serve as a measure of the degree of evolutionary allometry, or the extent to which adult body proportions vary with increasing absolute body size within the tribe. If we use as a criterion the average of the

absolute values of the deviations from unity in all the relations investigated, it is easily shown that the degree of allometry is least in the terrier tribe and greatest in the pug and bulldog tribes.

It should be pointed out that where a tribe is represented by only a few breeds, it is doubtful whether much significance can be attached to the particular values obtained for the constants. This is especially true of the curves characterizing the pug tribe, which for the most part include only two points, and which are therefore to be regarded as merely crude approximations to the true curves, that is, to those which would result if all the breeds of the tribe were included.

Moreover, in the absence of data on a greater number of breeds, certain parts of the present classification must be considered as tentative. For example, it is possible that the distinction between the bulldog and great Dane tribes might prove to be invalid on the inclusion of breeds of intermediate size, such as the English mastiff and Bordeaux dog. At the same time, the results obtained in the analysis of the zygomatic width / basilar length relation indicate that the pug tribe is very probably a valid group, even though it comprises only two of the modern breeds investigated.

Wagner (1930) finds that for a number of relationships the two bands representing his Groups *a* and *b* intersect at approximately the point representing the wolf. He regards this as supporting the theory that the wild ancestral type of dog corresponded to *C. lupus* in size and form. The present results bear out Wagner's finding only in the case of the snout length / cranial length relation. In other respects the wolf generally conforms either to the terrier tribe or to the great Dane tribe. These results, despite the fact that they differ somewhat from Wagner's, appear to support the above theory equally well. More specifically, the wolf may perhaps be regarded as corresponding to the ancestral type of

these two tribes, and the remaining tribes as having been subsequently derived from them.

The relation between b and α . If for any of the relations listed in Table II the values of $\log b$ for the different tribes are plotted against the corresponding values of α , the points thus obtained fall approximately along a straight line with negative slope. That is to say, the values of b and α in each case conform approximately to a decreasing exponential function of the form

$$b = Be^{-r\alpha}$$

where B and r are constants. This expression has previously been found to be applicable in similar instances by Hersh (1931, 1934), by Lumer (1939b), and, in a modified form, by Hamai (1938).

The values of B and r for each curve were determined graphically. Those of r are roughly equal, varying only between 4.61 and 5.37 with an average value of 4.87. On the other hand, B varies considerably, its lowest value being 14.8 and its highest 120. The curves obtained for the different relations are thus a set of approximately parallel lines.

The values of b and α for different relations within a tribe do not yield a distinct curve when plotted as above, but instead fall within a fairly wide linear band.

In a previous investigation (Lumer, 1939a), the author has shown that an inverse relationship between the constants b and α is generally to be expected as a consequence of dimensional connections between them, and that under certain circumstances the relationship would assume the exponential form given above. It was pointed out further that the occurrence of such a functional relationship does not in itself give any clue to the significance of the constants, since the dimensional considerations involved are independent of the particular biological application of the power function in which they appear. Hence it is doubtful what significance is to be attributed to the approximate conformity of the constants in the present case to a decreasing exponential

function. It is, however, of some interest to note that the conformity does exist here as well as in the previous instances.

Ontogenetic relative growth. From the data of Becker (1923), which consist of measurements on the skulls of thirty German shepherds of various ages, the values of the ontogenetic relative growth constants were determined for five different relations. These values are given in Table III, together with the values obtained for the same relations in the terrier tribe, of which the German shepherd is a member.

TABLE III

VALUES OF LOG b AND a , TOGETHER WITH THEIR STANDARD ERRORS, FOR CERTAIN SKULL RELATIONS IN THE TERRIER TRIBE AND FOR THE CORRESPONDING ONTOGENETIC RELATIONS IN THE GERMAN SHEPHERD

Relation	Terrier Tribe		German Shepherd	
	log b	a	log b	a
PW/PL ...	0.442 ± 0.010	0.694 ± 0.005	0.443 ± 0.023	0.687 ± 0.013
SL/JL ...	-0.459 ± 0.030	1.106 ± 0.014	-0.418 ± 0.020	1.094 ± 0.010
OW/BL ...	0.072 ± 0.011	0.781 ± 0.005	0.179 ± 0.028	0.738 ± 0.013
ZW/BL ...	0.524 ± 0.012	0.670 ± 0.006	0.116 ± 0.059	0.843 ± 0.027
PL/BL	-0.296 ± 0.012	1.015 ± 0.005	-0.114 ± 0.010	0.939 ± 0.004

Comparison of the two sets of values shows that they do not differ significantly for the first two relations. That is, in these instances the ontogenetic curve for the German shepherd effectively coincides with the group curve for the terrier tribe. On the other hand, for the last three relations the differences are well above the borderline of significance.

It should be pointed out that these results are not entirely conclusive, since it is not certain that the measurements of Becker and Wagner are fully comparable. Moreover, in comparisons of this type the situation may perhaps be further complicated by differences in environmental factors, which are known to exert a marked influence on the shape of the skull (see Weidenreich, 1925). The difficulty is increased by the fact that the characteristics of individual breeds change with time as a result of changes in the basis of selection. It is obvious that

in comparing any two groups all these considerations must be taken into account.

Despite these drawbacks, it would nevertheless be of value to determine similarly the relative growth constants for other breeds, and to compare them with one another as well as with the constants for the group curves. However, no comparable data for any other breed are available.

DISCUSSION

The results of the present investigation are essentially in agreement with those of previous studies. Hersh (1934) found that for certain skull relations in the titanotheres all the species investigated form a single curve or band, while for others they form a set of distinct curves, each comprising a single genus. Similarly Robb (1935a) observed that all types of fossil and modern horses fall within a single band with reference to the muzzle length / total skull length relation, while for the relation between length of splints and length of cannon bone (Robb, 1936), two distinct bands are produced, one including the four-toed and three-toed forms and the other the one-toed forms. The results obtained by Hamai (1938) for the relations between height, length, and breadth of the shell in a number of species of snails are in the main similar to those of Hersh.

There arises first the problem of interpreting curves of this type in terms of ontogenetic processes. It is obvious that such group curves will result whenever the ontogenetic relative growth curves of all the members of the group coincide, that is, whenever all have the same values of b and α . The constants of the group curve will of course be the same as those of the ontogenetic curves.

The present results may be most simply explained on the hypothesis that the converse is also true, namely that if for a given relationship the points representing the adults of different types form a single curve, then it

follows that the ontogenetic curves are the same in all the types in question. Some evidence in support of this hypothesis has been presented by Hersh (1934), Robb (1935b), and Lumer (1939b). Hamai (1938), on the other hand, raises certain objections to it, maintaining primarily that the group curves in general represent merely averages of the ontogenetic curves.

To test its validity in the present case would require the determination of the relative growth constants for a number of breeds in each tribe, a procedure for which the available data are very inadequate. The results obtained for the German shepherd seem to militate against the hypothesis, since in three of the five relations investigated the ontogenetic curves deviate significantly from the corresponding group curves. However, as has already been pointed out, these results are not conclusive, and the question should be regarded as still open.

In any event, even if differences in b and α of the ontogenetic curves exist between members of the same tribe, we should not expect them to be nearly as great as those between members of different tribes. Hence it seems justifiable to regard each tribe as a group of breeds relatively homogeneous with reference to genes controlling relative growth, and differing primarily in those affecting adult body size. Or, stated in other terms, the process of evolution within a tribe involves primarily mutations affecting only absolute body size, whereas the evolution of a new tribe involves also mutations producing marked changes in relative growth.

It may be remarked that Wagner (1930) reaches essentially the same conclusion. He distinguishes between size-determining and form-determining genetic factors, and maintains that with respect to the latter, Groups a and b offer different "material" on which the former may operate.

The group curves in the present case differ in one respect from those obtained by Hersh (1934) and Robb (1935a, 1936). The evolution of both the titanotheres

and the horse is characterized by a progressive increase in body size; hence on these curves successive points represent successive stages in time. This is not true of the domesticated dog, for here there has been no such orthogenetic trend. On the contrary, the process of artificial selection through which most of the modern breeds have originated has involved selection for smaller body size as much as for larger. This does not, however, affect the interpretation of the group curves, since the difference lies only in the mode of selection. In both instances the group curves may be regarded as representing the effect of selection for different adult body sizes with no pronounced change in the mechanism of relative growth. Thus the term "evolutionary relative growth" (or its equivalent, "evolutionary allometry") need not, as Hersh (1934) implies, be restricted to those cases in which an orthogenetic trend has occurred. Moreover, the concept may be applied to extant as well as to extinct groups.

From the interpretation presented above, it follows that within a tribe the larger breeds recapitulate in their development (at least approximately) the body proportions of the adult stages of smaller breeds. Such a recapitulation has been found by numerous investigators to occur not only in the dog, but in other domesticated mammals as well (for partial reviews of the extensive literature on this subject, see Becker, 1923; Weidenreich, 1925; Sommer, 1931). These observations have led several investigators to view the condition in dwarf forms as a retention of juvenile characters resulting from developmental arrest.

This concept has been elaborated particularly by Hilzheimer (1926) in his theory of *Verjugendlichung*, according to which arrested development with consequent dwarfing and retention of juvenile proportions in the adult stage occurs not only in dwarf forms, in which it affects the entire body, but also in larger forms, in which only isolated parts are affected. Bolk (1926) has at-

tempted to apply essentially the same idea to man in his fetalization theory, in which he characterizes man as a "sexually mature primate fetus." Bolk's theory has been criticized by Hilzheimer (1927), who maintains that it is valid only for some parts of the human body, and not for the body as a whole.

From the point of view of the present investigation, it is sufficient to point out that where an actual case of retention of juvenile proportions occurs, it is simply a consequence of a decrease in adult body size with no significant change in the course of relative growth. Whether this may properly be called a developmental arrest is debatable, but this point need not be discussed here. On the other hand, where the forms of the same absolute body size exhibit differences in proportions, these must have been achieved through differences in relative growth, or through a cessation of growth in the parts in question before the rest of the body has ceased growing. Unless the latter process is known to have occurred, and this seems at first glance a rather unlikely event, it can not be said in such a case that one form represents a fetal or juvenile stage of another.

The classification of the domesticated dog into allometric tribes proposed here is essentially an extension of that proposed by Wagner (1930). Both of these, however, differ radically from earlier systems, which are based solely on similarities of adult proportions.

The system of classification which has been most widely accepted is that originally elaborated by Studer (1901), which is based on the conception of each of the main prehistoric types as the ancestral form of a group of modern breeds. This classification, in a modified form proposed by Hilzheimer (1933), is briefly as follows:

1. *C. f. palustris* (spitz, terriers, pinschers, etc.)
2. *C. f. intermedius* (hunting dogs)
3. *C. f. matris optima* (sheepdogs)
4. *C. f. inostranzewi* (mastiffs, bulldogs)
5. *C. f. grajus* (greyhounds).

In the present system of classification, the first three and to some extent the fourth of these groups are combined in the terrier tribe. Similarly Wagner (1930) places all the first three in his Group *a*, on the grounds that the differences between them can be ascribed entirely to differences in absolute body size, which are not an adequate basis for racial distinctions. This contention is fully borne out by the present results. Furthermore, as we have seen above, there is some doubt as to whether the bulldogs may be regarded as having been derived from *C. f. inostranzewi*.

Schäme (1922), on the basis of measurements on individual skull bones, has attempted to classify all dogs into two fundamental types, namely, the *decumanides* type, characterized by short frontals and maxillaries and long parietals and represented by the great Dane, and the *veltrides* type, characterized by long frontals and maxillaries and short parietals and represented by the German shepherd. Götze and Dornheim (1926) sought to verify Schäme's hypothesis statistically by investigating the frequency distributions of certain size indices. They interpreted the resulting frequency curves as being bimodal, and concluded that Schäme's two fundamental types represent ancestral forms, of which most modern breeds are hybrids. The validity of such an interpretation, however, is extremely doubtful, for their curves are very irregular and may in most cases be equally well interpreted as having four or five modes, or, for that matter, as being unimodal. It is evident, therefore, that there is no valid basis for Schäme's system of classification.

A somewhat similar system has been proposed by Marchlewski (1930b), who distinguishes three main types—*C. leineri*, *C. matris optimae*, and *C. decumanus*. The first includes the greyhounds, the second the sheepdogs and similar forms, and the third the hounds and gun-dogs. This classification is based on an analysis of skull proportions by the method of least differences, in which

the sum of a number of measurements, each expressed as a percentage of basilar length, is compared in different breeds. Those breeds which differ least in this respect are considered to be most closely related. Since it is conceivable that two skulls differing in many proportions may nevertheless give nearly equal values of the sum used as a criterion, even despite possible correlations between the measurements, the validity of this method is somewhat questionable. Moreover, this approach, like the others discussed above, suffers from the defect of taking into consideration only similarities in adult proportions and neglecting the fact that differences in them may be purely a consequence of differences in absolute body size.

In the present investigation an effort has been made to clarify the relationship between evolutionary changes in body form and the underlying ontogenetic processes. In the determination of body proportions in the vertebrates, an important factor is the activity of the endocrine glands, particularly the thyroid. Thus, as Weidenreich (1925) points out, it has long been known that a high correlation exists between the distribution of goiter and that of brachycephaly in man. Weidenreich also adduces evidence to show that thyroid deficiency is involved in the occurrence of "pugheadedness" (*Mopskopfigkeit*) in domesticated animals. More recently, Dye and Kinder (1934) have demonstrated that thyroidectomy in young dogs, through its effect on ossification of the cartilage bones, results in marked brachycephaly in the adults. The work of Stockard (1931, 1932) also shows a definite association between endocrine function and adult body proportions in dogs. Both Weidenreich and Dye and Kinder suggest that hypothyroidism may be an important factor in the determination of racial types.

The foregoing discussion demonstrates clearly the need for more extensive investigation, particularly of the ontogenetic relative growth in the different breeds, and of the correlation between endocrine function and

relative growth. It would be of especial value to ascertain, by suitable breeding experiments, the relative roles of environmental and genetic factors in producing changes in ontogenetic growth relations, a point on which there has been considerable confusion. It is largely this confusion, it may be noted in passing, which has led Weidenreich and many other investigators, notably Hiltzheimer and his students, to adopt a thoroughly Lamarckian point of view in dealing with the problem of the origin of new racial types. Investigations along the lines suggested, together with a more inclusive analysis of adult types, should go far toward providing a solution of the general problem of the relation between ontogenetic development and the evolution of racial types.

SUMMARY

The law of allometry, $y = bx^a$, was applied to measurements on the skulls and limb bones in twenty-eight modern breeds of dogs. The data conform to the equation, yielding in some cases a single curve including all the breeds, and in others two or more distinct curves. On the basis of these results, the various breeds of dogs may tentatively be classified into six *allometric tribes*, each characterized by the fact that for any given relation all its members form a single curve conforming to the law of allometry. This conformity is interpreted as indicating that the breeds within a tribe are genetically relatively homogeneous with respect to ontogenetic relative growth. According to the available evidence, the proposed classification is valid also for ancient and prehistoric types of dogs. The European wolf, however, does not fit into any one tribe. For the same relation in different tribes, the values of b in all cases conform approximately to a decreasing exponential function of a .

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SHORTER ARTICLES AND DISCUSSION

DO HABROBRACON FEMALES STING THEIR EGGS?

IN a recent article Spotkov (1938) refers to an early paper by Hase (1922) on sterility in the parasitic wasp *Habrobracon juglandis* (Ashmead). Hase, who at first called his species *H. brevicornis* (Wesmael) but later (1925) corrected this, found that some eggs laid by either mated or unmated females failed to hatch, in one experiment 188 among 317. Spotkov found that when eggs were collected immediately upon being laid and fixed during the first hour of development, almost all (895 among 900) showed normal cleavage. He states that "this is in remarkable contrast to the number of 'sterile' ova obtained by Hase" and suggests that the "'sterile' ova, as described by Hase, may be produced by accidental puncturing of the eggs by the sting during oviposition." The implication is made, in this comparison of cleavage observations and final hatching percentages, that all or almost all eggs undergoing normal early cleavage will hatch if uninjured by external factors. Spotkov states that the accidental puncture causes loss of protoplasm, but he records no observations as to frequency of puncturing. About 60 per cent. of all eggs would have to be punctured to account for Hase's results.

To the author who has worked with *H. juglandis* for twenty years this idea of the stinging of eggs by mothers came as a surprise. Other investigators were questioned, but none had observed stinging of eggs or thought of it as a possibility.

In 1923 a series of preliminary observations were made on hatchability of eggs from several stocks. Females were transferred to fresh host caterpillars every twenty-four hours, and there was, therefore, ample opportunity for injury to eggs by stinging. Females of all types produced about the same number of eggs per day, so chances for stinging were the same for all. Hatchability was about 80 per cent. for unmated and for outcrossed females, 49.1 per cent. for close-crossed (those mated to related males). Bostian (1935), using females from one stock only, confirmed these results. He found hatchability to be 81 per cent. from unmated females, 83.2 per cent. from outcrossed and 66.8 per cent. from close-crossed. It is difficult to think of any mechanism whereby the type of male to which a female has

been mated would influence the number of eggs which she stings.

In passing, it may be observed that the higher mortality from close-crosses is due to lower viability of those diploid zygotes which are potentially male, produced only in such crosses, and that this phenomenon obtains within the species wherever tests have been made. Inaba (1939) has recently reported it also in *H. pectinophorae* Watanabe.

The author has watched the laying of more than twenty-five hundred eggs of *Habrobracon*. When they were laid singly each was removed at once and placed on a paralyzed host caterpillar, but when a female laid several in rapid succession they were not disturbed until she had finished. Toward the end of the experiment Spotkov's paper appeared, and during the last part of the work an accurate record was kept on 508 eggs laid by twelve unmated females and every possible case of injury recorded. Twenty-two (4.33 per cent.) were described as having been touched by the sting. Four of these were undoubtedly injured, since minute oöplasmic extrusions appeared; one hatched and died without feeding, the other three did not hatch. Of the remaining eighteen, eight became adults, one died as a pupa, three as larvae and six failed to hatch. The mothers had been heavily irradiated in this experiment, so that a large percentage of these eggs would not have hatched in any case.

A recent experiment planned for another purpose happens to give a more convincing answer to this question. In order to determine time of death of eggs which, for various reasons, do not hatch, eggs were collected at intervals and placed in the mineral oil "Nujol," where development proceeds normally and conditions can be clearly observed. Numerous observations were made and records kept.

Certain general facts should be mentioned here. An occasional female lays many sterile ova. Data on eggs of such exceptional females are not included in general summaries. There is likewise a tendency on the part of many females to lay "shells," apparently filled only with fluid, which dry up very quickly and become brittle. In one series the percentage of such "shells" was 2.26 for the first ten days of life of the females and 12.06 for the second ten days. Some females produced none, although all were from the same inbred line. If these "shells" result from stinging, it must be concluded that stinging females grow more awkward in their old age and differ innately in degree of awkwardness.

These "shells" are likewise omitted in computing hatchability ratios.

It would be logical to suppose that the percentage of eggs stung would be dependent upon (1) length of time females were left with host, (2) number of eggs on a single host and (3) number of females ovipositing on a given host during a given time. Data are classified from these points of view in Table 1.

TABLE 1

HATCHABILITY PERCENTAGES FOR EGGS OF DIFFERENT GROUPS OF FEMALES. TOTAL NUMBERS OF EGGS ON WHICH PERCENTAGES ARE BASED ARE GIVEN IN PARENTHESES. DATA ARE ARRANGED ACCORDING TO LENGTH OF TIME FEMALES WERE ALLOWED TO REMAIN WITH THE HOST, NUMBERS OF EGGS LAID ON SINGLE CATERPILLARS, AND NUMBERS OF FEMALES OVIPOSITING IN A SINGLE CONTAINER

Females		Eggs on host						Females per container	
		Time			Number			One	Two
No.	Group	1-8 hours	1-24 hours	1-48 hours	1-10	11-20	21-30		
10	33 unmated	94.58 (309)	89.32 (740)	89.43 (246)			88.27 (162)		90.77 (1355)
16	33/11-o mated		90.70 (355)	93.54 (263)					
16	33/11-o unmated				88.30 (265)	88.63 (209)		88.47 (564)	

For eggs collected within eight, twenty-four and forty-eight hours of laying, differences between hatchability percentages of twenty-four- and forty-eight-hour groups from both types of mothers are not statistically significant. The percentage of hatchability for the eight-hour group, however, is significantly higher. This may be due to less chance of being stung or to the fact that the firm yolk-filled freshly laid eggs are less easily injured than embryos when the transfer to the oil is made. Hatchability percentages for eggs grouped according to the number of eggs per host do not differ significantly, nor do those for the containers with one female and with two:

Hatchability percentages obtained by the oil method are higher than those reported earlier in this paper, because this method allows for greater accuracy of observation. In the previous work the difference between eggs and larvae counted directly on the host was taken to represent egg mortality, and minute larvae which died immediately after hatching were often missed. The same sixteen 33/11-o females were used for data from mated and unmated in Table 1. After several days of egg-laying they were

mated and further collections made. Hatchability percentages after mating are those expected from outcrosses, which these matings were.

Each of the 1,226 eggs collected from the 33/11-0 females was examined especially for evidence of having been stung. Twelve (0.97 per cent.) were recorded as "possibly injured." They were somewhat wrinkled but contained what appeared to be normal yolk in contrast to the dry "shells" described above. They did not hatch. The mothers in this experiment were young, and only seven "shells" were laid. If it is believed that these two groups represent stung eggs, then 1.54 per cent. (19 among 1,226) were stung.

A final observation of considerable importance in this discussion is that almost every egg which fails to hatch continues development after it has been removed from the container with females and dies at a later embryonic stage. This agrees with Spotkov's observation that almost every egg undergoes cleavage.

From these studies the author concludes either (1) that females sting eggs very rarely or (2) that stung eggs can continue development after such injury and (3) that normal cleavage and even blastoderm formation are no indication of ultimate hatching.

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THE REPRODUCTION OF *DUGESIA TIGRINA* (GIRARD)

Dugesia tigrina (synon. *Euplanaria tigrina*, *Planaria maculata* Leidy) has been known for a long time to reproduce in some habitats exclusively by fission and in others mainly by egg capsules.¹ I have kept animals from both a "sexual locality" and "asexual localities" in laboratory culture for six years and have

¹ W. C. Curtis, *Proc. Boston Soc. Nat. Hist.*, 30: 515, 1902.

attempted to analyze the factors that might control the processes of reproduction. Both lots were exposed to conditions as nearly identical as possible, each lot in several parallel cultures. All the cultures retained their characteristic kinds of reproduction through several generations: the animals from asexual localities continued to undergo fission, while those from the sexual habitat showed regular annual seasons of sexuality and laid cocoons. All attempts to induce the development of genital organs in the asexual animals by changing external factors (amount of food, temperature) were unsuccessful. From these observations I concluded that there must be two distinct physiological races of *D. tigrina*, a sexual and an asexual one.²

In a recent paper, L. H. Hyman³ reports her observations on the occurrence of sexual and asexual animals of *D. tigrina* in natural habitats. She noticed that sexual specimens are found in habitats with considerable movement of water, as in streams or along the shores of lakes and large ponds, where they are exposed to the action of waves. On the other hand, small ponds and other habitats with quiet water contain only asexual worms. Hyman believes that "moving water is the essential environmental factor in the development of sexuality in *D. tigrina*." She considers the annual recurrence of sexual reproduction in my sexual cultures as no decisive evidence for the existence of a genetically distinct sexual race, "for it might well be that after worms have once attained maturity in nature (under the influence of running water) they might continue to be sexual in laboratory culture, at least to some extent."

Hyman's theory disagrees with the fact that not only animals collected in nature attained sexuality, but likewise those hatched from cocoons in the laboratory and then grown, under usual laboratory conditions, in still water. Hyman finds it difficult to evaluate this result "in the absence of exact data." As detailed records had been omitted from my former paper,² I wish to supplement here the data concerning this particular question.

Seven lots of cocoons, deposited in laboratory cultures at various times, were isolated in separate dishes and kept under observation. In all these dishes the animals matured, and in the next breeding seasons laid egg capsules. The cultures gave in every respect the impression of normal sexual cultures, and no decline

² R. Kenk, *Biol. Bull.*, 73: 280, 1937.

³ Libbie H. Hyman, *Trans. Am. Micros. Soc.*, 58: 264, 1939.

in the production of cocoons was observed. Table I gives the time of hatching and that of full maturity for some of these lots.

TABLE I

Animals hatched in the laboratory	became mature and laid cocoons
III/30 to V/29, 1933	I/29 to VI/18, 1934, and again XII/24, 1934, to V/2, 1935
III/24 to VI/4, 1934	VII/23 to IX/3, 1934, and XI/29, 1934, to VI/17, 1935
VI/11 to VII/16, 1934	XII/31, 1934, to VI/21, 1935
VIII/13 to IX/14, 1934	II/7 to VI/21, 1935

Though in the sexual race sexual reproduction occurred in regular annual cycles, fissions were occasionally observed in single individuals during the period of sexual inactivity which coincided with the warmest season of the year. The animals which had fissioned (head and tail pieces) were segregated from the cultures and observed separately. They preserved, however, in every way the characteristics of the sexual race, becoming sexually mature and laying cocoons in the following breeding season.

Fig. 1 represents a typical pedigree of seven cultures, showing the times of sexual reproduction and of fissioning. Four cultures (II, III, IV and VII) were started from cocoons and attained sexuality in the next breeding seasons (in culture III, cocoons were laid also in the late summer, which is rather exceptional). Animals grown from fission products (cultures V and VI) likewise became mature in due time.

From these data it is evident that in the material I worked with (collected in the pond behind the Episcopal Church in Falmouth, Massachusetts) sexuality was not the result of exposure to moving water. It was apparently determined by "internal" factors, *i.e.*, by the genetic constitution of the animals.

I had suggested to Dr. Hyman that the particular ecological distribution of the sexual and asexual animals in nature, the first being found in quiet and the latter in moving water, might indicate an active preference of the two races for their respective habitats. She considers this interpretation impossible, since sexual and asexual animals sometimes are found close together in the same habitat. In this case, however, it remains to be proven by experimental culture that the asexual animals are not simply young individuals of the sexual form or else that the

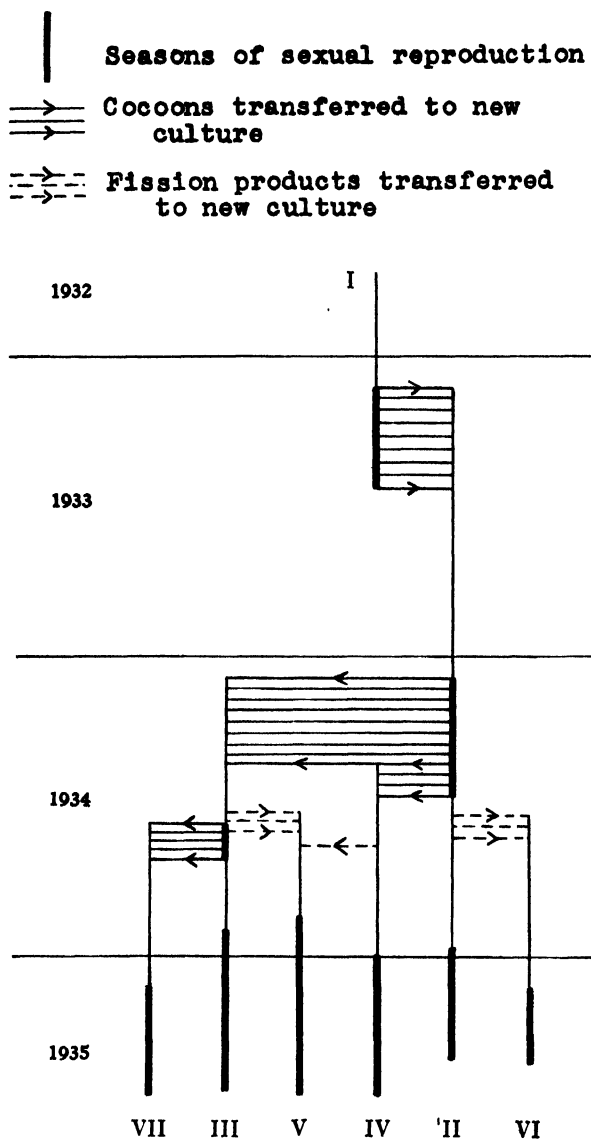


FIG. 1. Pedigree of seven cultures of the sexual race of *Dugesia tigrina*.

separation of the two forms by their habitats is not always possible.

Finally I wish to point out that the conclusions drawn in my paper² are, until further investigation, necessarily limited to the

material studied (collected in Virginia and Massachusetts). It is indeed possible that *D. tigrina* in other localities exhibits different physiological characteristics.

Hyman³ announces that she hopes to test whether asexual "races" of *D. tigrina* can be made sexual by means of running water. It will be interesting to see the results of her experimentation.

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TRANSLOCATION BETWEEN SPERM AND EGG CHROMOSOMES AS EVIDENCE THAT BREAKAGE PRECEDES UNION

THE problem of the mechanism of induced gene rearrangements has aroused much controversy. At an early stage of attacking this problem two alternatives presented themselves according to Muller (Painter and Muller, 1929). According to one of these, which is more closely analogous to the crossing-over mechanism, the involved chromosomes—non-homologous in the case of translocations—come in contact with or in close proximity to each other in some way, such as lying across one another, and both break at the point of contact or point nearest as a result (direct or indirect) of one common disturbance, *e.g.*, one single ionization. This is essentially the same as the theory advanced by Serebrovsky (1929) and later adopted by Dubinin (1930). The other alternative, which was advocated by Stadler (1932) and later adopted, in part at least, by Muller (1935) (see Dubinin and Khvostova, 1935; Kirssanow, 1937; Muller, 1938, 1939), is that the breaks occur independently as a result of independent causes, *e.g.*, independent favorable ionizations. According to the second scheme, the breakage ends may reunite where they parted—the result being the reconstruction of the original chromosomes—or they may unite in new ways, giving rise to gene rearrangements.

The first of the two alternatives mentioned above is called the "contact hypothesis" and the second the "breakage-first hypothesis." A combination of both of the above alternatives is also conceivable. That is, some gene rearrangements might be produced by one common ionization, while others were produced by independent ones; this might, in fact, even apply to

different breaks involved in the same case of multiple-break rearrangement.

It was the hope of helping toward the settlement of this question that two years ago a large-scale experiment was started, by Muller and the author in collaboration, on the frequency of translocations with two dosages of x-rays, one four times the other. A ring-shaped-X-chromosome as well as a non-ring-X-chromosome were used to facilitate a comparison between the frequencies of double-and multiple-break translocations.

The data obtained show clearly that the total frequency of translocations varies approximately as the 1.5 power of the dosage for the doses used (1000*r* and 4000*r* units). The exponent of the dosage to which the frequency is proportional is definitely higher than one and lower than two. This is to be expected on the breakage hypothesis only. (This work is now being prepared for publication.)

In the experiment mentioned above, for the non-ring-chromosome males from the wild-type, Oregon K stock was used. For the ring-shaped-X-chromosome, Beadle's stock was used in which the males have the so-called Xc² ring and the females have attached X's homozygous for yellow. The males from both stocks were x-rayed and mated to virgin females of composition $\bar{y}\bar{y}$ bw e ey, i.e., having attached X-chromosomes homozygous for yellow and their three pairs of autosomes homozygous for brown, ebony and eyeless, respectively; these females also have a Y-chromosome. These matings were carried on in mass cultures in bottles. The F₁ males heterozygous for bw, e and ey and so phenotypically wild-type for Xc²P₁ males had carried the normal alleles—were backcrossed individually in small vials to virgin $\bar{y}\bar{y}$ bw e ey females. Their progeny were examined for the presence of translocations according to the segregation of characters shown, as explained in the following key:

Translocations involving	Segregation of characters
X-II . . .	all males bw ⁺ ; all females bw
X-III	" " e ⁺ ; " " e
X-IV	" " ey ⁺ ; " " ey
II-III	All flies either bw e or bw ⁺ e ⁺ ; no bw e ⁺ or bw ⁺ e
III-IV	" " " e ey or e ⁺ ey ⁺ no e ey ⁺ or e ⁺ ey
II-IV	" " " bw ey or bw ⁺ ey ⁺ ; no bw ey ⁺ or bw ⁺ ey
No translocation ..	All possible recombinations present of pairs of genes considered two at a time.

Among 11,569 fertile backcross cultures in which Xc^2 males had been used, one gave a progeny showing an unexpected type of segregation of characters. All the males were ebony and the great majority of the females were non-ebony.

A translocation between sperm and egg chromosomes seemed the most likely explanation. This case must have come about in one of the following alternative ways:

(1) An interstitial translocation involving the paternal ring-shaped-X-chromosome and the maternal third chromosome. According to this hypothesis it would be more probable that the ring-shaped X in the irradiated spermatozoon from which the translocation containing F_1 male was derived was broken at two points by the treatment, and that the third chromosome carrying the gene for ebony in the egg which this spermatozoon fertilized happened to have broken spontaneously (not as a result of the x-ray treatment) at one point. Conceivably, however, the ring was broken in one place and the third chromosome in two. After fertilization, the interstitial fragment—deleted from the chromosome having two breaks—became inserted in the gap produced by the breakage of the other chromosome, while the remainder of the chromosome with two breaks joined together again (deletion). The probable direction of exchange above mentioned would be indicated by the presence of a few (2) exceptions among the female progeny (ebony females).

(2) A translocation involving the paternal third chromosome carrying the normal allele for ebony, and the maternal Y-chromosome. The translocation-containing F_1 male will thus have the gene for ebony on his third chromosome and the gene for the normal allele of ebony on his Y-chromosome as a result of the translocation, and so he will be phenotypically non-ebony. But when he was backcrossed to an $\bar{X}X\bar{Y}$ female of the same stock as his mother, the daughters will get the e^+ -containing Y-chromosome, hence being non-ebony, while the sons will get the e -containing third chromosome, being ebony. A few exceptions, arising by non-disjunction between the X and the translocational Y, would not be unexpected here.

Less likely possibilities considered were:

(3) A translocation involving a treated paternal Y and the treated paternal third chromosome. In this case it would have to be assumed that the irradiated spermatozoon carried both X- and Y-chromosomes, due to the occurrence of non-disjunction

either in its immediate formation or in its ancestral cells. Then as a result of the x-rays, a translocation took place involving its Y-chromosome and its third chromosome carrying the normal allele for ebony. The translocation-containing F_1 male will thus have two Y-chromosomes, one of them carrying the normal allele for ebony as a result of the translocation. But that would mean that the ring-X-chromosome would segregate freely with either of the Y-chromosomes, resulting in a larger number of exceptional—non-ebony—as well as numerous ebony females. This result did not obtain, however.

(4) A translocation involving both the Y- and the third-containing-chromosomes of the female, which occurred independently of the x-rays. Then in the F_1 male a crossing-over in the nucleus ancestral to the whole of the gonads took place between the paternal ebony-containing third chromosome and the maternal translocational third chromosome, as a result of which the Y-chromosome became connected with the e^+ , which was thus transferred to the daughters of the backcross, while the sons got the e -carrying third chromosome. This is obviously an extremely unlikely hypothesis.

Accordingly, the only plausible explanations are the two first mentioned. The decision between them came when males were mated to non-ebony females with attached X's containing $y\ v\ f\ car$ and the males derived from this cross—which, as expected, were non-ebony—were backcrossed to homozygous ebony females (of stock called "rucuca") having separate X's. The offspring showed no sex-linkage of ebony, a fact which proves that their ring-X-chromosome had not been involved in the translocation. This finding rules out the first explanation and thus shows that the correct is the second, namely, a translocation involving the paternal third chromosome and the maternal Y-chromosome.

Spontaneously occurring translocations are so exceedingly rare that the chance of their occurring in any given experiment is practically negligible, and so it is extremely probable that the radiation played some part in the origination of the present translocation. Nevertheless, on the breakage-first hypothesis, the frequency of individual breaks, occurring spontaneously, would be far higher than that of the translocations themselves, since the latter would require at least two breaks at once, a coincidence whose frequency would be only of the order of the square of the individual-break frequency. Thus it is on this

hypothesis, by no means surprising, that there should have been a spontaneous break in one of the maternal chromosomes which underwent recombination with one of the numerous induced breaks that occurred in the paternal chromosomes. That is, the irradiation of the sperm would have greatly facilitated the occurrence of just such a translocation as that which we observed.

On the other hand, on the contact hypothesis, the present translocation would have either had nothing to do with the radiation at all—being one of those exceedingly rare cases that happen spontaneously—or else we should have to invoke for its explanation some kind of considerably delayed after-effect of the irradiation on the paternal chromosomes, other than the effect of breakage, yet an effect of such a nature as somehow to communicate itself from them to the maternal chromosomes. All this seems much more improbable than the breakage explanation, with the premises of which the finding falls naturally into line.

It should further be noted that this case not only serves as an evidence that chromosome breakage precedes union, but also provides further evidence that the union of the broken pieces need not occur during the spermatozoon stage but can be, or must be, delayed until after fertilization. This (in its stronger form) is a conclusion at which Muller, Makhijani and Raychaudhuri (see Muller, 1939) had already arrived on the basis of other results, showing the independence of the frequency of translocations induced by treating spermatozoa from the time factor. Thus, the fact that our results lead to this idea as well must be regarded as increasing the strength of our interpretation as a whole.

In conclusion, we may draw attention to the fact that this case forms the only one yet recorded of a very special type, *i.e.*, a translocation between the paternal and maternal chromosomes. This had for several years been an object expected by those who favor the breakage hypothesis. The author is informed that it was for the obtaining of such a case as evidence for the breakage hypothesis that, while on a visit to the Institute of Genetics, Moscow, Altenburg had proposed the institution of an experiment in which both males and females should be irradiated and bred immediately. Circumstances have till now prevented the carrying out of such an experiment, but the present case has unexpectedly provided some of the desired evidence. It would still be desirable, however, to find the frequency of this phenomenon in control material and in material in which the eggs alone, the sperm alone and both gametes were treated.

SUMMARY

In the course of our work on the relation of translocation frequency to dosage of irradiation and on the relative numbers of double- and multiple- break translocations, one translocation was found between the third chromosome derived from an irradiated spermatozoon and the Y-chromosome derived from the non-irradiated egg which that spermatozoon fertilized. That the Y had not been present in the spermatozoon was shown by breeding tests of the original translocation-bearing individual, since these showed it to contain but one Y-chromosome. Unless we adopt the less probable view that the irradiation had nothing to do with the production of the translocation or that it gave rise to a long-delayed after-effect whereby translocation by contact was brought about, we must conclude that this case arose as a result of union of pieces of a third chromosome broken by the x-rays in the spermatozoon with pieces of a Y-chromosome that happened to have broken spontaneously in the egg. Thus the case argues strongly in favor of the breakage theory of structural change. The hypothesis adopted is not rendered improbable by the rarity of spontaneous structural changes, since on the present view surviving rearrangements require the coincidence of two spontaneous breaks, and this coincidence must be far rarer than the occurrence of only one break.

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REGENERATION¹

INTRODUCTION

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SELF-REPAIR characterizes all protoplasm. It is a fundamental perquisite in adjustment to the environment provided by a violent world. Self-repair may be occult, involving de-toxification and restoration of intra-cellular components, or patent, involving reconstruction of lost structure. In the latter we may distinguish rather sharply between structural stoppage of the wounded region which preserves more or less vicariously the wholeness of the organism, and the restitution of the lost structure in a form both structurally and functionally complete. The latter, regeneration in its strict sense, is presented for consideration here. Except to the student of the phenomenon, the dramatic character of regeneration is often overlooked. For not only is the process *dramatic* in many cases, but so obvious in its outward manifestations and so secretive in its fundamental processes as to excite the imagination and to defy analysis. For example, the forelimb of a salamander is cut off. Immediately and before the unaided eye the wounded animal proceeds to demonstrate with startling boldness many of the major problems which preoccupy the student of developmental physiology. Cells migrate and are mobilized at the wound area,

¹ A symposium presented at the meetings of Section F, American Association for the Advancement of Science, at Milwaukee, Wisconsin, June 21, 1939, and dedicated to the late Professor Henry Van Peters Wilson, 1863-1939, professor of zoology in the University of North Carolina.

ingesting moribund and destroyed tissue. Under some as yet unknown influence, cell divisions begin and a white blastema quite readily visible to the unaided eye very soon appears. Subsequently, this blastema elongates, and presently the several tissues of the normal limb become differentiated. With unvarying accuracy the elongated structure assumes the characteristics of the member removed. The phenomenon of polarization becomes evident; symmetry appears, and growth continues until within several months the newly formed limb attains the functional and structural equivalence of the former limb. Few materials in all biology demonstrate with such completeness and on such a large scale cell division, growth, differentiation, symmetry, polarization and the establishment of function. The observer is challenged by this performance, yet the controls operating in the large-scale events taking place under his most careful scrutiny are so elusive that thus far they have not been stated with convincing clarity. True, it is possible to perform certain measurements and to describe the process in terms of change of size and change of condition with time. It is also possible to describe with considerable accuracy the order in which the several tissues and parts of the new limb appear and to state the obvious fact that these structures have the high respiratory rate and high susceptibility of young tissue. But as yet there has been laid before us too little that convincingly demonstrates the physiological controls upon which development depends.

What is true of our knowledge of the fundamentals in the regeneration of the limb of the salamander is also too largely true of our knowledge of regeneration in other forms of animal and plant life. But effort that has been perennial since the time of Spallanzani has produced an enormous literature from which may be culled much that is helpful.

Regeneration may be considered both as a problem in itself and as a technique or approach to the study of more general problems. Valuable indeed is the study of the

potentials and course of regeneration in the various levels of animal complexity, but we are not greatly aided in the solution of its fundamental processes by such data. For it is often found that the capacity for regeneration varies very widely between rather closely related groups. For example, the capacity for regeneration in the polypharyngeal flatworm, *Phagocata gracilis*, may be described as high, fragments as small as one-sixteenth oftentimes regenerating the whole. But in *Procotyla fluviatilis*, a member of a genus so closely related that the exact position is still a matter of dispute, regeneration potential is confined to the anterior third of the animal; section in more posterior regions results in a simple form of wound healing.

Similarly important are the changes in regenerative capacity with changes in age of the animal. Although the generalization may be made that the capacity for regeneration decreases with age, there are certain confusing exceptions. For example, in certain ascidians cleavage is highly determinate, isolated blastomeres being incapable of reconstituting the whole, yet the adult is capable of extensive regeneration.

Most attractive and suggestive are the evidences and data on fundamental biological problems obtained in studies on regenerative phenomena, for in every case of regeneration the investigator is faced by an amazing variety of problems. What consequences of injury or of isolation are responsible for the mobilization of the repair mechanism? What induces the accompanying cell divisions? What cells are involved—specific regenerative cells or tissue cells that in the uninjured normal animal perform quite other functions? Is differentiation thus a reversible process and do the cells involved in formation of the regenerate de-differentiate prior to reorganization of the lost part, or are residual undifferentiated or plastic cells solely responsible for repair? What are the influences that induce differentiation of the several new tissues in the regenerate? What controls the spatial arrange-

ment of regenerating parts? By what means is the new or restored part limited in growth and organization to replacement of the lost part? For only occasionally does hyper-regeneration occur. And towering above all these and other problems of detail is the immensely greater problem of integration of the individual.

Since the time of Plato much has been said concerning the integrating mechanisms that convert an aggregate of individuals into an organism of higher order with properties and potentialities not predictable in the qualities of its members. The Gestalt theory is one modern form of the concept. Too often metaphysical influences have been prematurely called into being to explain individuation and too often these are flavored with vitalism. The integrations of animal societies have recently been brilliantly reviewed by Emerson. It is, of course, unthinkable that the mechanisms of integration of the cells that compose the biological individual are identical with those operating in a group of individuals. Nor is there any logical necessity for assuming that integrating influences which confer wholeness are identical in different types of animals.

In form development Wilhelm Roux drew the exact geometrical centre of the problem of integration in his generalization that the developmental fate of a cell is influenced by its position with reference to other cells. Whitman likewise in his remarks on the inadequacy of the cell theory calls attention to the importance of the spatial relations of cells. Tremendously valuable contributions illuminating the mechanism of organismic integration have been made in recent years by the experimental embryologists.

But nowhere else is the principle of individuation more strikingly expressed than in regeneration. In miniature, reconstitution in Protozoa expresses the organization factors. In the re-association of cells of the lower invertebrates integration and rise of the new individual are necessary complements. The organization of a new and complete animal from a fragment of a flatworm or an annelid implies again the principle of individuation.

At this point regeneration blends with the phenomenon of agamic reproduction. Briefly stated, agamic reproduction involves the property of reconstituting a complete individual from a single cell or group of cells and implies the property of developing the leading, dominant, or organizing element which assumes control both morphologically and physiologically, in the integration of the regenerate. Thus the study of regeneration oftentimes becomes an important approach to the study of the physiological states responsible for agamic reproduction, so common in plants and widely employed among animals. Here again the nature of the organizing and maintaining influences in individuation becomes the goal.

A mechanistic interpretation of organizing action requires that the implements of individuation be physiological in character. Although we have recently been discouraged by R. S. Lillie's scholarly analysis arriving at the conclusion that we can not hope to know the nature of the organizing principle, we have been encouraged by the adduction of certain evidences of knowable controlling factors. One, and perhaps the more widely held view, is that organismic control is established and maintained by reason of the diffusion of specific organizing substances arising as the result of specific metabolisms of organizing or inducing centers. Much confirmatory evidence has been obtained by experimental embryologists, yet there is no general agreement as to the fundamental nature of such substances nor as to their action on cells and tissues brought under control. What is still more important and fundamental in such a concept of the nature of induction, namely, the accounting for the apparent fact that organizers attain a specifically qualitatively different metabolism from that of subordinate regions, is, in general, omitted from the theory. As yet, regeneration has afforded no direct evidence of the existence of organizing substances. If they are present in an isolated fragment of a planarian, we must account for their origin at one particular region of the piece, namely,

that portion which later regenerates or reconstitutes the head. Since all regions of such a piece are capable of producing a head and all are subjected to injury and isolation, but only the anterior end reproduces the head, it becomes exceedingly difficult to apply the organizer concept implemented by organizing substances to head regeneration in planarians.

A second interpretation of the nature of the organizing action relates it to primary quantitative differences in protoplasmic activity between dominant and subordinate regions. Considerable evidence in support of this interpretation has accumulated from studies on regeneration, particularly in Coelenterates, Planaria and Annelids. Some small portion of that evidence will engage attention here.

All major biological problems are embedded in huge masses of literature representing the attempts at solution of many investigators. Regeneration, because of its inclusion of so many more general problems, has a particularly voluminous literature. The restrictions of a single treatment impose the necessity of confining attention within certain bounds. Logically, the first consideration must deal with the nature and behavior of cells engaged in the regenerative process. Secondly, can the morphogenic properties of regenerative cells be controlled, and can the nature of the process be inferred from the nature of the action of the externally applied experimental influence? Thirdly, in how far are similar observations and controls discernible in regeneration in one of the higher invertebrates, the Annelids? Fourthly, do single cells, in this case Protozoa, exhibit the same sorts of organismic morphogenic controls as do the Metazoa?

THE HISTOLOGIC BASIS OF REGENERATION AND REASSOCIATION IN LOWER INVERTEBRATES

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BEFORE proceeding, I should explain what I shall mean by "organismal" factors in regeneration as contrasted with "histological" factors. By histological factors I mean, of course, the changes whereby the various types of cells regenerate the new individual or part. By organismal factors I mean the physiological conditions in the organism as a whole, or in certain parts of the organism, that may be significant in relation to the histological and other changes of the regeneration. Thus, cell divisions, migrations, differentiations or de-differentiations are histological factors; polarity, symmetry and metabolic gradients are organismal factors. These two aspects of the regenerative processes may be distinguished, however intimate may be their relationships in the final analysis. Regeneration can be studied from either of these aspects, at times to the disregard of the other; there can be no complete understanding of the process until we have adequate knowledge of what the individual cells are doing of themselves and under the influence of the masses of cells wherein the organismal factors in some manner reside. This complete analysis should be kept in mind as the goal, however distant its attainment may seem. Although my own concern has been principally with the histological factors of regeneration, I have never been unmindful of the organismal aspects of the problem.

When one studies the histological factors the role of the mass of cells is inevitably considered, since the mass of the regenerate conditions the places where cells divide, where they migrate, where and how they differentiate. When one studies the organismal factors, the cells can be forgotten for the time being, if one is thinking not of

cells but of regions of the organism in their relation to the whole. There are, in fact, many papers dealing with the organismal factors of regeneration, which might have been written in the absence of any knowledge concerning cells and in which the word cell may not even appear. This is not said in disparagement of such work; the investigator has fixed his attention upon this aspect of the problem and proceeded accordingly. Indeed, I think the organismal attack upon regeneration, as upon the problems of ontogenetic development, has been much the more fruitful in recent decades. Under existing techniques there are certainly more things one can do with an animal as a whole or with pieces of an animal containing many cells, than with its cells individually. Moreover, the histological changes are often easy of determination as compared with determination of the organismal factors conditioning such changes. But in the last analysis it will be necessary for the investigator to "know his cells" as well as his organism.

Certain of the histological data with respect to regeneration in the lower invertebrates may now be presented in summary statement. Since the Porifera have almost no specialized parts that can be removed and then restored, and since growth in sponges so overlaps with restorative processes, it is difficult to speak of regeneration in the Porifera in the same sense as in other phyla. Moreover, the restorative powers of many sponges appear to be limited, although some genera can be reproduced from cuttings and the development of gemmules in fresh-water sponges has certain resemblances to regeneration. The conspicuous phenomenon in sponges is the reassociation. Like the processes in sponges that can be called regenerative, reassociation seems to occur only in certain species. Hence, the problem of contrasting regenerative powers appears even in these lowest invertebrates. The histological study of reassociation indicates that dedifferentiation and subsequent redifferentiation into cells of other types do not occur. The

only cells that survive in numbers in the reunion masses are the choanocytes, which lose their collars and flagella temporarily but become normal choanocytes again in the newly forming mass, and the archæocytes, one type of which, the nucleolate cells, can form all the other cell types including choanocytes, while another type, which includes smaller and more active cells, forms only the new epidermal membranes. The cells of the epidermal membranes in the original sponge and the cells of the middle region seem to disintegrate and thus to have no part in the restoration. It is difficult to be sure what happens, but according to the late H. V. Wilson, whom we honor by this symposium, (1932, p. 166), "it has yet to be proven that sponge cells ever dedifferentiate into a regenerative, embryonic condition, although the dissociation phenomena in hydroids lend some support to the view that this is possible"; to quote again from Wilson, "the retention by cells of their essential nature, even after complete morphological dedifferentiation, is illustrated by the dissociated collar cells." In sponges, with their indefinite bodily organization and manner of growth, the histological aspect of reassociation is uppermost. There is not much to be made of the matter from the organismal aspect where the individual is so diffusely organized, yet there is a problem. On the histological side, cells that can be studied in fixed material from the reassociation masses and from the normal sponge, and living reassociation masses that can be followed day by day in what might be called "natural" tissue cultures, offer tempting possibilities for investigation. The limitations are the difficulties in interpreting the types of cells and their grouping in the sponge, even before dissociation, and the low level of sponge organization. Aside from the problem of reassociation, I may express the opinion that the dissociated cells and the reunion masses of sponges present material for the study of morphological and physiological problems that is not yet appreciated.

In cœlenterates, the histological changes of regeneration are not easily followed. The traditional conclusion

that the interstitial cells of such a cœlenterate as the Hydra represent an undifferentiated stock from which all the other cell types arise, both in regeneration and in reproduction and growth, has been questioned of late years. McConnell (1933) describes abundant mitoses in the epitheliomuscular cells. Strelin (1929), on the other hand, has found in studies upon irradiated material what he regards as a complete correlation between the occurrence and activities of interstitial cells and the power of regeneration in *Pelmatohydra oligactis*. Kanajew in three publications (1926) first concluded that the basis for regeneration was the transformation of interstitial cells into other cell types. His later study (1930) did not substantiate this finding; he then concluded that buds and regenerating parts were formed principally at the expense of neighboring differentiated cells, as shown by the transplantation of parts that had been vitally stained as well as by histological study. The relative potencies of ectoderm and endoderm in the regeneration of cœlenterates have been examined without conclusive results. Gilchrist (1937) has found that pieces of ectoderm from a syphistoma of Aurelia can regenerate complete new individuals; Papenfuss and Bokenham (1939) find that neither ectoderm nor endoderm alone can regenerate a new Hydra. The development of an effective technique of vital staining, of irradiation, of tissue culture, or the like, might lead to more consistent results if there be consistency in the phenomena.

The data are much more extensive from the organismal standpoint. The individual is simple in the cœlenterate but its differentiation is sufficient for the purpose. Cymorpha, for example, has been an endless inspiration to Professor Child. What has been said in general regarding histological *versus* organismal studies upon regeneration can be repeated with respect to regeneration in cœlenterates. If the organismal attack has shown greater possibilities under existing techniques, this does not mean that the histology of the regeneration need not

be known, nor that the fundamental explanations reside elsewhere than in the cells.

In nemerteans, the comprehensive investigations of Coe have laid a foundation for organismal studies. The nemertean tissue presents difficulties for the histological approach. Nevertheless, Coe (1934) has made something of the histological changes. With respect to the contrasting powers of regeneration in closely related species, he ventures only the comment that these "may be dependent upon differences in the extent of distribution either of the activating agent or of the regenerative cells."

The turbellarians, notably the planarians, are more favorable for histological study; and they have served as a perennial source of material for the organismal attack. For many years investigators of the histological changes have commonly agreed that the so-called "formative" or "regeneration" cells of the mesodermal region are the immediate source from which new parts arise during regeneration. The disagreement is concerning the origin of these formative cells. Some maintain that the formative cells originate by dedifferentiation, others that these cells are a persistent stock (*cf.* Fig. 15, Curtis and Schulze, 1934). It has also been maintained that the contrasting powers of regeneration in certain genera of planarians may be related to the relative abundance of formative cells whatever their origin.

In my own studies and those of my students this problem has been attacked by the technique of irradiation in the hope that the riddle of the parenchyma, as well as that of the formative cells, might be brought nearer to solution. My conclusions regarding the effects of irradiation with x-rays are supported by the studies of Weigand (1930) upon the effects of irradiation with radium in several species of planarians. Briefly, these results of Weigand and myself are as follows: It appears that exposure to x-rays and to radium inhibits regeneration in planarians, as shown by the reduction or by the absence of scar-tissue and by the proportionate restriction of the

regeneration. When such non-regenerates are examined histologically, it is seen that the formative cells are destroyed in numbers related to the degree of irradiation and of failure in regeneration. It is almost impossible to find any such cells in individuals whose regeneration has been completely inhibited. One observes in the fixed material that the formative cells show signs of disintegration soon after the irradiation and that they may have disappeared completely before other changes become apparent. The similar effect of such irradiation upon cells with embryonic potencies will be recalled. Observing that the formative cells have thus been destroyed in non-regenerates, it is only natural to conclude that the failure to regenerate is due in part, if not wholly, to the elimination of these cells, which are known to be of prime importance for the regeneration. The weakness of this conclusion that the failure to regenerate is conditioned, at least in part, by the destruction of formative cells lies in the fact that it has not yet been possible to produce planarians in which the power of regeneration has been completely inhibited and which continue alive and otherwise normal for a long period as in the case of the annelid *Tubifex*, noted in a subsequent paragraph. The planarian which has received an exposure of x-rays sufficient for complete inhibition of the regeneration dies some weeks later with external symptoms resembling those produced by various toxic agents. One must admit that there may be general physiological effects induced by the irradiation as well as the effect that is recognizable in the destruction of formative cells—that the inhibition may be only partially conditioned by the effect upon the formative cells. Also, it can be argued that the primary effect is of a general physiological nature which conditions first the death of the formative cells and later the death of the planarian as a whole. It can be argued as well, in the absence of experimental evidence, that the primary effect lies in the differential susceptibility of the formative cells to the rays and that toxic effects of the disintegrating material thus produced are responsible

for the general physiological changes which end in the death of the entire animal.

In an investigation intended primarily to determine whether *Euplanaria dorocephala* shows a differential susceptibility to x-rays as to many other agents, Strandskov (1934) has examined the effects of x-rays upon planarian regeneration. He finds a differential susceptibility shown by planarians to various agents in accordance with the observations of Child and others. He notes, p. 585, that the planarians affected by the irradiation "regenerated in most cases some, if not all, the tissues that they had lost as the result of the effects of the x-rays," although he says he would not have expected this result on the basis of my observations (Curtis, 1928) which he cites. Again, p. 584, Strandskov states that, "whether or not 'formative cells' of the type described by Curtis are present in *E. dorocephala* the writer can not state, but if they are, they do not seem to be especially susceptible to x-rays, at least of the kind emitted by the tube under the conditions given." In view of the well-known occurrence and functioning of such cells during regeneration in many species of planarians, it would be surprising if they are not similarly present and functioning in *E. dorocephala*. The evidence of their destruction, following exposure to x-rays, may be easily observed by one who studies the necessary histological preparations. In a later paper Strandskov (1937), as stated in his summary, p. 20, seems to find more evidence for an inhibition of regeneration in relation to x-ray exposure, although "most of the irradiated pieces regenerated some tissue." He says further that "there is a decrease in the maximum degree of regeneration with each increase of dosage; also with each increase in interval between the irradiation and the time of the cutting of the pieces." Again, he states, that "although it was found that x-ray dosages can be given which will prevent pieces of *Euplanaria* from regenerating any tissues immediately following x-ray irradiation, no piece which had permanently lost the ability to regenerate was obtained and kept alive for any considerable length of time."

It seems to me that nothing in these results of Strandskov (1934 and 1937) is incompatible with the hypothesis that formative cells are responsible for the histological changes during regeneration, and that these cells, being more susceptible to the rays, are destroyed by the irradiation, as they are seen to be when the experimental material is examined histologically. Since varying exposures to x-rays destroy varying numbers of these cells, the cases in which the power of regeneration persists can be explained as related to the survival of a certain number of formative cells; restoration of full regenerative powers would be expected when a sufficient number of formative cells had arisen from these surviving cells by division. If formative cells arise by dedifferentiation as well as by multiplication of existing formative cells, the explanation of the restored power of regeneration is even more reasonable in terms of these cells.

A problem with respect to the formative cells of planarians is their apparent migration. Since the evidence for this migration rests upon the interpretation of series in fixed material, it presents the limitations inherent in such evidence. For example, one must consider the possibility that the pressure of contraction in the fixation of the worm may change cell shapes and thus produce in sections cell outlines suggestive of migration. If one could observe the formative cells moving actively in tissue cultures or when bits of the planarian are teased in a saline solution, the observations upon fixed material would be confirmed to this extent. I have never seen these movements in the living cells although shapes are observed like those found in sections. With respect to the evidence specifically: The scar tissue, or blastema that is visible externally, is composed principally of these cells which later differentiate into the new parts. Internally, a new pharynx forms in a similar manner from an accumulation of formative cells. However, there is no cell division, in these accumulations of cells, sufficient to account for the appearance of cells in such numbers. Mitotic cell divisions do appear in numbers in neighbor-

ing regions of the parenchyma; and between regions of cell division and cell accumulation the formative cells are frequently observed to be shaped as though in migration. The time relationship in the series of fixed specimens confirms this interpretation.

Some years ago, I hit upon a type of operation and subsequent regeneration that presents what I regard as a crucial test of migration by the formative cells and also a test of dedifferentiation in this instance. By a special technique, one fourth to one third of the free end of the pharynx is removed without other injury to the planarian. With the possible exception of the head region, or of the genital complex, the pharynx is the most specialized region of the animal; there seem to be virtually no formative cells within its boundaries. If dedifferentiation occurs to produce the blastema that eventually appears at the cut end of the pharynx, one might expect that this dedifferentiation would be clearly recognizable in the absence of neighboring formative cells. What happens is that nothing happens at the tip of the pharynx until formative cells arrive upon the scene by migration. The cut end of the pharynx remains a raw stump until the necessary formative cells reach this exposed surface, apparently by migration from the parenchyma of the body and through the length of the pharynx. From the blastema composed of cells assembled in this manner the new epithelial, muscle and other cells are formed.

The relationships involved in this series of events include formative cells in the parenchyma in such numbers as might be expected in view of the physiological state of the individual. For example, worms from stocks active in fission have many formative cells and much mitosis in these cells; food is also a factor. As noted, there are virtually no formative cells in the pharynx itself. After the pharynx is cut there is perhaps an increase in the number of mitoses among the formative cells in the region of the parenchyma near the anterior end of the pharynx, although this can not be determined with certainty; soon there appear formative cells shaped and

located as though migrating toward the pharynx. Next, there is a noticeable accumulation of these cells against a barrier-like group of muscle fibers which radiate from the lumen at the base of the pharynx and presumably function in retraction of the extended pharynx, or perhaps in the peristaltic contractions by which food is pumped through the lumen. Only when the formative cells have thus accumulated in numbers at the barrier does the migration through the pharynx begin. It appears that this movement occurs principally within a few hours, because worms showing maximum numbers of these cells within the old pharynx are not so readily found as worms showing stages of accumulation at the barrier, or worms with stages of differentiation in the blastema at the newly forming tip. The time at which any one stage occurs is somewhat variable, but the sequence is definite. One finds cells in numbers at the barrier, in numbers within the pharynx, or in numbers at the tip, never in numbers at all three places. Typically, in the earlier stages after the cutting there are many formative cells at the barrier and there are no such cells at the tip; later, there is a maximum number of these cells within the pharynx and lesser numbers at the barrier and at the tip; finally, there is a fully formed blastema with many differentiating cells at the tip and almost no formative cells at the barrier or within the older part of the pharynx.

The appearance of the newly arrived cells at the beginning of differentiation is suggestive. Cells are seen applied to the old epithelium of the outer surface of the pharynx and of its lumen as though becoming attached upon arrival and differentiating under the influence of the old epithelium and of exposure at the surface. As the blastema becomes covered with the new epithelium, muscle fibers, gland cells and parenchyma begin to appear. The only interpretation of this sequence of stages seems to be migration as indicated. It may be that less differentiated cells such as those of the gut dedifferentiate on occasion, in other parts of the planarian, after the manner described by Steinmann (1926 and 1937); in

this case of the pharynx, I conclude that the cells of this organ remain unchanged and that regeneration is initiated and consummated only by the formative cells after they arrive at the tip.

Understanding of what may be happening histologically is easier in the turbellarian than in the coelenterate. The difficulties in the turbellarian are the questionable distinction between formative cells and the apparent syncytial structure of the parenchyma, and the possibility that dedifferentiation occurs. I have remarked that most of the investigators who have studied the histological changes in planarians have concluded that the parts formed during regeneration arise directly from the cells commonly known as the regeneration or formative cells, whatever may be the source of these cells. It may be noted also that such cells give rise to the germ cells and to other parts of the reproductive organs when these are formed, and that such cells can be recognized in very early stages of development; formative cells are, in fact, much like the late blastomeres of the embryo (*cf.* Fig. 15, Curtis and Schulze, 1934).

With respect to the histology of regeneration in annelids, to which attention has been devoted in my laboratory, some of the cases that have been described are perhaps as clear as any in the animal kingdom. In *Tubifex tubifex*, the neoblasts, earlier described by Kreckler (1910 and 1923) and others, were studied by Stone (1932 and 1933) with the aid of irradiation. Exposure to x-rays inhibits the formation of these neoblasts and also the cell divisions of the ectodermal and endodermal epithelium that are necessary for part of the regeneration. The irradiated worms from which posterior segments have been removed heal the wound and form a characteristic stump but nothing more. Again it seems reasonable to suppose that the known effect of x-rays upon cells with embryonic potencies is the primary factor involved and that inhibition of the action of such cells or their destruction is primarily responsible for the inhibition of regeneration. I say "inhibition" of neoblasts as well as de-

struction, because neoblasts are not clearly recognizable as a cell type among the peritoneal cells of the septa on which they make their appearance in the regenerate. The histology of regeneration in *Tubifex* lacks one item that might be desired, since the neoblasts are not found in normal worms as a distinct cell stock; it is only that peritoneal cells produce neoblasts upon proper stimulation. In this respect the formation of neoblasts, though more spectacular, resembles the origin of regenerative cells from the ectodermal and from the endodermal epithelium of the *Tubifex*. With respect to securing an individual "castrated" against regeneration, the case of *Tubifex* is perhaps as good as can be expected. In Stone's cultures some of the irradiated individuals survived 147 days without regeneration, or as long as the non-irradiated controls. Other irradiated specimens were cut at intervals during comparable periods, but formed only a new posterior stump without regeneration. The controls, on the other hand, regenerated after each of several cuttings during such periods.

Our advancing knowledge regarding the histology of regeneration in the several phyla has been summarized in various publications during recent years by Goetsch (1929), Curtis (1934) and Stolte (1933). Difficulties of the kinds cited in the foregoing comments upon the cellular changes in lower invertebrates are encountered in higher forms. The recent work of Thornton (1938 and 1938) upon the regeneration of the forelimb of *Amblystoma* has interested me particularly. This investigator makes a case for dedifferentiation that I find convincing. One must admit from the standpoint of genetic and cytological theory that if all cells contain all the chromosomes and hence all the genes, and if differentiation is principally a matter of the cytosome, then there is good theoretical reason why dedifferentiation may occur. Also, there is abundant evidence supporting the contention that, in the course of development, time and place make cells what they come to be, as Roux contended years ago.

One thing that troubles me with respect to Thornton's work is the correlation of his results with the established fact that irradiation by x-rays prevents regeneration in these same salamanders. If dedifferentiation occurs and if there is no stock of cells with embryonic potencies, the explanation is not so simple as the mere destruction of such a stock. There seems to me need for a complete study of the histological changes of regeneration in these salamanders as they may be related to the known effects of x-rays upon the regeneration. Exposure to x-rays inhibits, or at least affects profoundly, the regenerative processes in many known cases from cœlenterates to vertebrates. Current work by DeBoer (1940) shows that the regeneration of feathers, like that of hair, is checked by x-radiations. What does all this mean histologically? When we have the same kind of an effect by the same agency in so many widely separated cases the action of similar factors is naturally assumed, as the working hypothesis to be examined critically while investigation proceeds. In this case the hypothesis first indicated is the destruction of embryonic cells and the disturbance of mitotic cell division, both of which are known to occur in cells with embryonic potencies, following exposure to x-rays and to radium.

To conclude, in addition to reviewing certain investigations upon the histology of regeneration in lower invertebrates, I have emphasized my conviction that knowledge of what individual cells do in the course of regenerative development is necessary for any comprehensive understanding of this process. Investigation necessarily begins with the gross morphological changes and proceeds on the one hand to histological study and on the other to study of the subtle physiological states of the organism as a whole or of its parts. So far as possible the data from these two lines of study should be correlated. The organismal phenomena present more points of attack with our present techniques and perhaps will always be the more significant. Yet it is my conviction that the ultimate answers lie with the cells.

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THE ENVIRONMENTAL CONTROL OF REGENERATION IN EUPLANARIA¹

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ZOOLOGISTS long ago recognized the remarkable capacity of pieces of certain planarians to regenerate or reconstitute into whole animals. It has been, however, only within the last three decades that an experimental analysis of the process has been undertaken in earnest and certain factors controlling regeneration have been brought to light.

Before discussing the subject of environmental control of regeneration in Euplanaria, it should be mentioned that a large number of experiments involving thousands of animals have shown that *E. dorotocephala* (as well as a number of other species) normally possesses a gradient in physiological activity which is highest at the anterior end of the animal and gradually diminishes posteriorly until it reaches the posterior zooid region. It has likewise been shown that while all levels have the capacity to regenerate a normal head and to reorganize into a complete animal, pieces of a certain size and from certain levels of the physiological gradient fail to do so because of other correlative factors present in the piece. Our problem deals with the control of such correlative factors as well as the direct control of regenerating tissues through the use of various environmental agents.

Let us now pass on to some of the pertinent observations and experiments in which *E. dorotocephala* has been the chief experimental animal.

THE HEAD-FREQUENCY PROBLEM

Transverse pieces of *E. dorotocephala*, if allowed to reconstitute in well-water in the laboratory, may develop any of the following head-types if the size and level from

¹ Since the presentation of this paper Hyman (1939) has pointed out that the generic name *Dugesia* has priority over *Euplanaria*.

which the piece is taken is controlled (Child, 1911a, 1911b, 1911c, 1912, 1914a, 1914b, 1916, 1920, 1924):

(1) *Normal*—with triangular head, auricles at lateral margins and two symmetrically placed eyes.

(2) *Teratophthalmic*—with triangular head and auricles in normal position but with eyes showing all degrees of approximation to the median plane from two distinct eyes with pigment connected up to complete cyclopia.

(3) *Teratomorphic*—with head more or less rounded in outline and with auricles more or less anterior and showing all degrees of approximation to the median plane up to a single median auricle and with a single, or apparently single, median eye.

(4) *Anophthalmic*—with head rudimentary, without eyes and with or without a single median auricle.

(5) *Acephalic*—with head completely absent.

When the anterior zooid of 14–16 mm worms is divided into fourths (exclusive of the head) it is found that the most anterior (*A*) pieces regenerate almost 100 per cent. normal heads. In the next most anterior (*B*) pieces the number of normal heads is decreased while the number of inhibited heads and forms in which the head is completely absent is increased. Such inhibition in head formation is even more noticeable at (*C*) levels, while the most posterior (*D*) pieces may be almost completely anophthalmic and acephalic.

The frequency with which the different head-types appear at the different levels has been termed the head-frequency of that level for pieces of a particular size. In order to arrive at a value for the comparison of head-frequencies of different levels of worms of the same and different lots, the "Head-Frequency Index" has been devised (designated as "mean" by Child and Watanabe, 1935). This is arrived at by giving to each head-type a number which approximates its degree of inhibition (*Normal* = 100; *Teratophthalmic* = 80; *Teratomorphic* = 60; *Anophthalmic* = 40; *Acephalic* = 20). The actual number of each head-type for any particular level is then

multiplied by the number corresponding to its degree of inhibition and the sum of all divided by the total number of worms regenerating from that level. It can readily be seen that the head-frequency index of pieces from *A* level, which regenerates almost 100 per cent. normal heads, will approach 100 while the index of *D* level, which regenerates chiefly acephalic forms, will approach 20. Many experiments have shown that if the size of pieces from posterior levels is increased, the head-frequency increases, and if smaller worms are used, the head-frequencies of pieces involving equivalent fractions of the entire animal are decreased.

Under normal conditions there seems to be at least two different factors which determine whether or not short transverse pieces will develop a head. The first of these has to do with the antero-posterior physiological differential of the animal. When the anterior portion of the worm is removed a new head will grow on the posterior portion, but the rate at which the new head will regenerate will depend upon the level of section (Watanabe, 1935a). When the section is made at an anterior level of the first zooid the rate of head regeneration is more rapid than when the section is made at a more posterior level of the same zooid.

Although the rate of head development is different at different levels, all levels can and do regenerate normal heads. The fact that the various medially inhibited head-types do appear in short transverse pieces from posterior levels of the anterior zooid points to other factors in operation in short pieces which are absent or reduced when only a single section is made. The production of inhibited head-types in short transverse pieces seems to be due to some factor or factors arising at the posterior cut surface of the piece which may last for several days or during the early stages of tail formation. Evidence has accumulated which indicates that this head-inhibiting factor may be largely nervous in character (Buchanan, 1922, 1923; Child and Watanabe, 1935; Watanabe, 1935b)

and is probably conducted to the head-forming region by way of the ventral nerve cords.

Experimentation has shown that if the head-inhibiting factor be blocked or eliminated, or the head-forming cells near the anterior cut surface be given a start in the reconstitucional process (*i.e.*, by delaying posterior section for twelve hours or more), short pieces from any level may be caused to regenerate normal heads. It seems not unlikely that the reason short pieces from *A* levels regenerate normal heads while more posterior pieces of the same size regenerate inhibited heads or no heads at all is because the more active head-forming regions of the anterior pieces may break from the control of posterior inhibiting factors while the less active head-forming regions of more posterior pieces are unable to do so. But since we have no way for complete analysis, it may be that the head-inhibiting factor is stronger or more active in pieces from the more posterior levels.

THE CONTROL OF HEAD-FREQUENCY

Many different agents in the hands of a number of investigators have been used to increase the head-frequency in short posterior pieces. Such agents as dilute KCN (Child, 1916), chloretone, chloroform, chloral hydrate, ether, ethyl alcohol (Buchanan, 1922), caffeine (Hinrichs, 1924), strychnine (Miller, 1937), H-ion, carbon dioxide, methylene blue, formic acid, acetic acid, propionic acid, and butyric acid (Rulon, 1936, 1937, 1938) when applied immediately after section for a given length of time and in sub-lethal dosages have been found to increase the head-frequency. Mechanical stimulation (Child, 1920), supersonic vibrations (Wiercinski, 1939), and an abrupt change in temperature from 20 to 29 degrees C. (Behre, 1918) have also been found to increase head-frequency.

On the other hand, the head-frequency of pieces from anterior levels and of long pieces can be decreased by allowing the pieces to regenerate in dilute solutions of

metabolic end products, ethyl alcohol, KCN (Child, 1911a, 1912, 1916), certain anesthetics (Buchanan, 1922), and caffeine (Hinrichs, 1924).

Presumably an increase in head-frequency can be brought about either by blocking, or in some manner decreasing the head-inhibiting action of the posterior cut surface, by differential recovery of the head-forming region from the effects of general protoplasmic depressants (Buchanan, 1923), or by directly stimulating the head-forming cells near the anterior cut surface. A decreased head-frequency seems to result from either an increased effect of the inhibiting factor or a decrease in the activity of the head-forming cells near the anterior cut surface.

Animals of the same species and of the same size but from different stocks do not always yield the same head-frequencies for similar levels. Likewise, worms from the same stock vary considerably from time to time as regards head-frequency. Animals collected in the winter may show a head-frequency that is considerably different from those collected in the summer. At any one time worms of the same size but from different localities may vary as regards head-frequency (Rulon, 1936). Evidently the physiological condition of animals prior to section is important in determining regeneration in pieces after section. This difference in physiological condition must have been impressed upon them by their different environments before section.

In this connection it is interesting to note that a Japanese species from near Sendai resembling *E. dorotocephala* gave practically 100 per cent. normal heads in anterior and posterior pieces of one-eighth the body length or larger while only in shorter pieces did inhibited heads appear. Animals from what appear to be the same species but from a different region (Kyoto) show a head-frequency similar to that of *E. dorotocephala* (Child and Watanabe, 1935).

Worms in different physiological condition respond differently to various external agents after section. In

general, pieces from animals which show a relatively high control head-frequency react more readily to such agents as CO_2 and high H-ion and show a greater increase in head-frequency than do those pieces which show an extremely low head-frequency in the controls (Rulon, 1936). On the other hand, pieces (one-fourth the length of the anterior zooid) from animals with a high-control head-frequency treated with M/100,000 KCN for 72 hours after section show a lowered head-frequency at *B*, *C*, and *D* levels while *A* level is unaffected. Pieces from animals with a low-control head-frequency but with the same treatment show a slightly lowered head-frequency at *A*, *B*, and *C*, levels while *D* level shows an increased head-frequency (Rulon, 1938).

When intact animals are treated in the laboratory with certain agents before section, the head-frequencies of both tests and controls are altered when pieces from these animals are allowed to regenerate in various solutions after section. Animals, treated with high concentrations of CO_2 for 10 days prior to section and the pieces following section allowed to reconstitute in well-water (Rulon, 1936), show a slightly increased head-frequency at *C* and *D* levels, a slightly decreased head-frequency at *B* level, while *A* level is unaffected. When pieces from such CO_2 -conditioned animals are caused to regenerate in carbonate-free well-water at a pH of 4.15 the head-frequency is much higher at all posterior levels than non-conditioned animals regenerating in the same test solution. Normally, an increase in the calcium concentration of solutions of high H-ion will antagonize the effects of the H-ion on the regeneration of heads in *Euplanaria* but if the animals are treated with CO_2 before section, only a partial antagonism to the effect of H-ion is caused by increased calcium. Pieces from CO_2 -conditioned animals treated with .00032 M propionic acid for 96 hours after section have head-frequency indices of 100 for *A* and *B* levels, 99 for *C* level and 97 for *D* level. The untreated controls in well-water had indices of 100 for *A* level, 66 for *B* level, 25 for *C* level and 22 for *D* level (Rulon, 1937).

In contrast to the effects of CO_2 in increasing the head-frequency, it has been found that intact animals from stocks showing a high head-frequency treated for six days with M/100,000 KCN show a considerably lower head-frequency in posterior pieces when sectioned and allowed to regenerate in well-water. It may be of interest that this effect can partially be antagonized by allowing the pieces to regenerate in dilute methylene blue (Rulon, 1938).

THE CONTROL OF THE GRADIENT IN RATE OF HEAD REGENERATION

It was pointed out above that any level in *E. dorotocephala* will regenerate a normal head if the posterior section is eliminated or the inhibitory factors arising at the site of posterior section blocked or decreased. It was also pointed out that the head regenerating on the posterior piece of a worm cut in two regenerates at a faster rate if the section is made immediately behind the auricles. The time for head formation increases as the section is made more and more posteriorly in the first zooid. This gradient in rate of head formation is one aspect of the physiological gradient so often demonstrated in these forms.

Certain experiments have been carried out testing the effects of environmental factors on the gradient in rate of head formation (Rulon, 1936). Pieces five-sixteenths of the length of the animal from just behind the auricles (X-pieces) and from the next posterior level (Y-pieces – five-sixteenths of the length of the animal posterior to X pieces) usually do not show the effects of posterior section and were used in these experiments. The rate of head regeneration is indicated by obtaining the mean time of first appearance of eyespots as seen under the compound microscope. In one experiment involving fifty animals, carried out at a temperature of 20°C . and from a stock showing a high head-frequency in the controls, it was found that the eyespots appeared on X pieces at approximately 3.6 hours before they could be detected on

Y pieces. In an H-ion concentration of pH 4.2 the rate of eyespot formation was delayed approximately 10.6 hours in *X* pieces and 6.3 hours in *Y* pieces. In other words, the difference in rate of eyespot formation for the different levels is practically obliterated by allowing the heads to regenerate under such inhibitory conditions. It should also be mentioned that with similar animals under the same conditions the head-frequency gradient is also practically obliterated.

In another stock showing a low-control head-frequency at posterior levels it was found that the *X* pieces regenerated eyespots approximately 13.7 hours before *Y* pieces. Treatment with H-ion at pH 4.15 inhibited both levels so that the difference was cut from 13.7 hours to approximately 8.1 hours. The head-frequency gradient of this stock under similar test conditions was somewhat flattened out but by no means obliterated. It appears in this last experiment that the physiological differential was so great between the different levels that it was only partially destroyed by the H-ion.

THE CONTROL OF BIPOLARITY

The production of bipolar forms, that is, those types which have regenerated a head at both ends of the section, is another example of the decrease or elimination of correlative factors within a piece by the use of external agents.

Bipolarity has been induced in pieces 1 mm in length from *B* levels and from the region of the fission zone in *E. dorotocephala* by increasing the H-ion of the solution in which the pieces regenerated (Rustia, 1925). The most effective agent yet used, however, in the production of bipolar forms has been strychnine (Miller, 1937). Pieces one-eighth of the length of the body in 16 mm worms from the region just posterior the mouth (*E* pieces) placed in M/100,000 strychnine for 16 hours and then returned to well-water for the remainder of the regenerating period yielded as high as 31.4 per cent. bipolar forms while no bipolars appeared in the control.

It has also been found that bipolars can be produced in this form in one-eighth pieces from *E* levels in well-water by delaying the time of posterior section. In one case a delay of 18 hours in making the posterior section resulted in approximately 26 per cent. bipolars. When pieces were submitted to the effects of M/500,000 strychnine as well as the delay of posterior section as many as 73.8 per cent. developed as bipolar forms.

The high frequency of bipolar forms from *E* level seems to be correlated with the fact that this is the region of the fission zone. The pieces from this region seem to be characterized by little physiological differential between anterior and posterior ends. This, together with the fact that strychnine seems further to decrease correlative factors, apparently permits both regenerating regions to become physiologically isolated from each other and each to become activated sufficiently to regenerate a head.

THE CONTROL OF THE SCALE OF ORGANIZATION

Perhaps the most interesting effect of environment in the control of regeneration in *Euplanaria* has to do with the alteration and control of the scale of organization. It was found some time ago that pieces of *Euplanaria* not only regenerated a new head and tail but also underwent a complete reorganization of internal structures. It was found that such structures as the pharynx and mouth arose in the newly reconstituting form in definite spatial relationship to the head.

When a portion of the body posterior to the anterior zooid is removed and allowed to regenerate in well-water at 20° C., the piece regenerates a new normal head and a pharynx which is somewhat anterior to its mid-region (Child, 1915, 1924). If the piece is caused to reconstitute under certain inhibitory conditions the head is smaller and inhibited, with the pharynx appearing more and more anterior in the piece as the degree of inhibition is increased. If, on the other hand, regeneration is accelerated by increasing the temperature the scale of organization

is increased in that the head is larger and the prepharyngeal region lengthened.

The position of the various posterior organs seems to be correlated with dominance of the anterior end or the steepness of the gradient. If the anterior end has a high activity the length of the animal dominated and controlled is greater than when the activity of the anterior region is lowered as in the case of differential inhibition by temperature. The scale of internal organization seems to be controlled, at least in part, by environment in the action of external agents through the gradient system. If the gradient is steepened by differential acceleration or recovery, the scale is increased. If the gradient is flattened out by differential inhibition, the scale is decreased.

The effect of external agents in the modification of the scale of organization might be shown in another way (Rulon and Child, 1937). Short transverse pieces (one-sixteenth of the length of the animal) from the region just behind the auricles are from a region of high physiological activity, and, because of this activity, reorganization often takes place on a larger scale. Since the size of the piece is limited, the only part forming is that part which is most active in the complete animal. We therefore have a high percentage of forms regenerating from this region which are little more than heads since such short pieces regenerate a large head but little if any tail or posterior end when regeneration takes place in well-water under controlled laboratory conditions.

When similar short pieces are subjected to M/100,000 KCN for 72 hours and then returned to well-water the scale of organization is found to be decreased in many cases in that the number of tailless or partial forms is decreased while the number of forms with inhibited heads but normal tails is increased.

CONCLUSIONS

No developing organism is ever entirely free of the influence of environmental factors and the plasticity of

planarian tissues makes these forms all the more susceptible to environment and environmental changes. Inhibitory environmental agencies may act directly on regenerating tissues as in the case of inhibited heads in anterior or long pieces or they may act indirectly in the blocking or eliminating of correlating factors residual in the piece or arising at the site of posterior section.

Such factors as physiological dominance and isolation and such phenomena as differential tolerance, recovery, inhibition and acceleration may play a determining part in the type of regeneration of planarian pieces taken from a gradient system and already possessing, at least in part, the polarity of the parent. *E. dorotocephala* will regenerate a normal head at all levels and from short pieces of all levels if correlative factors within the piece be controlled. Such factors may be at least partially controlled by environmental influences imposed on the piece either before or after section. When the head is present it seems to arise in spite of the piece and on appearing tends to dominate to a certain extent the reorganization of the piece into a normal whole.

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ASPECTS OF REGENERATION IN ANNELIDS

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THE annelids are of great interest for the problem of regeneration because they are among the most highly organized animals capable of complete regeneration. Consequently, regenerative changes can be more easily followed than in lower, less differentiated forms, and the counting of segments can be used as a quantitative measure. Since the leeches are incapable of regeneration, the discussion confines itself to the oligochaetes and polychaetes. No new data are presented but the paper attempts to organize and correlate the known facts of annelid regeneration.

Among the annelids, as in the planarians, all gradations in ability to regenerate completely, occur. The word completely is used advisedly since practically every oligochaete and polychaete is able to regenerate posteriorly. It is then the question of anterior regeneration which is of most interest in annelids. In both the oligochaetes and the polychaetes this power is present in varying degrees. Among the lower or microdrilous families of oligochaetes, as the Aeolosomatidae, Naididae, and Lumbriculidae, almost any piece, even of very short length, with the exception of the body ends, regenerates completely. Among the higher or megadrilous oligochaetes, sporadic cases occur whose powers of regeneration are nearly equal to those of the microdrilous genera as the earthworm *Perionyx* (Gates, 1927). But in general, among earthworms only anterior levels are able to regenerate a head, and this ability ceases in the neighborhood of the fifteenth to twentieth segments. The least powers of head regeneration are seen among oligochaetes of intermediate taxonomic position, as the families Tubificidae and Enchytraeidae, which are practically unable to regenerate a head or, at best, can replace only the few most anterior segments when these are removed. Thus, among the

oligochaetes, power of anterior regeneration is related to taxonomic position only in a very broad way, for it is difficult to see any difference in grade of morphological differentiation between the Lumbriculidae with very high powers of regeneration and the Tubificidae with practically no ability to reform the head.

Moreover, among the polychaetes still less relation exists between taxonomic status and head regeneration. The errant polychaetes, universally regarded as the more primitive forms, as the nereids, eunicids, and phyllodocids, are in general incapable of any head regeneration at all, and die after decapitation. In another errant family, the Syllidae, all degrees of anterior regenerative power occur (Okada, 1928), and many members have almost unlimited powers of replacing the anterior end as *Procerastea* (Allen, 1921). But it is among the most modified and highly adaptive polychaetes, the tube-dwelling forms, such as the sabellids, cirratulids and serpulids, that unexpectedly great ability to regenerate the anterior end is found.

Clearly, gradations in regenerative power can not be explained as resulting from gradations in general morphological construction. Whether histological characteristics furnish an explanation is considered later. I may state at this point, however, that I do not believe any acceptable explanation has been found for the wide variations in ability to regenerate the head, which exist within a group of animals. That forms with regular modes of asexual reproduction, such as fission or fragmentation, have high regenerative ability is a truism; but why some annelid families regularly undergo fission and others of like morphology can not regenerate at all is not thereby explained. Again, several workers have attributed the regenerative powers of the tubicolous polychaetes to their liability to damage by enemies; but such a teleological view-point does not constitute an explanation.

The details of head regeneration reveal certain interesting facts. The ability to regenerate at the cut anterior surface approximately the number of segments removed

is exceptional. The best case of this kind is the syllid *Procerastea* (Allen, 1921), in which apparently the exact number of segments cut off anteriorly is regenerated; numbers varying from 9-31 were observed. According to Okada (1928), another syllid, *Syllis gracilis*, belongs in the same category, and *Diopatra* also is stated to regenerate as many anterior segments as are removed, although cuts did not extend beyond the thirteenth segment (Pflugfelder, 1929). In another group of forms, the number of anterior segments regenerated increases in general with the number removed up to a certain level, beyond which no further increase is obtained and the number replaced remains approximately stationary or declines as the level of section is moved posteriorly. A case in point is the earthworm *Perionyx* (Gates, 1927) in which if six or fewer anterior segments are cut off, the exact number removed is replaced; when more than six, the number regenerated increases with, but is generally less than the number removed and at no level are more than ten to fifteen regenerated. Similar conditions exist in *Criodrilus* (Tirala, 1913) which regenerates thirteen to twenty-three segments, mostly fifteen to eighteen, when twenty to thirty anterior segments are removed.

These are the less usual results. In the majority of annelids only a small number of segments regenerates at an anterior cut surface, regardless of the number removed, and in many cases this head regenerate has a distinctive morphology setting it apart from the rest of the body. Such annelids may be regarded as possessing a definite head or cephalic region, behaving as a unit in regeneration. Among the microdrilous oligochaetes, this cephalic region is usually obvious, being differentiated from the trunk region by the lack of dorsal setae bundles and absence of nephridia and chlorogogue cells. Here the number of head segments is definite and only this number is regenerated in anterior restitution. The number is three in *Acolosoma*, five in the *Naididae*, except *Pristina* and its allies which have seven head segments, seven or

eight in species of *Lumbriculus*, three to five in the Tubificidae. Among lumbricid earthworms, the differentiation of a cephalic region does not show externally but is evidenced by the regeneration of a head of only three to five segments at an anterior cut surface. The most striking cases of cephalic organization are seen among the tubicolous polychaetes in such families as the Chaetopteridae, Sabellidae, and Serpulidae. Here the body is divisible into cephalic, thoracic and abdominal regions—each with well-marked morphological characteristics. The cephalic region bears various sensory organs and appendages and generally has reduced and modified parapodia; the thoracic region is also distinguished by various modifications of the parapodia and setae, and only the abdominal region bears typical parapodia.

It is characteristic of all such annelids with a definitely organized head region that regardless of the number of anterior segments removed, only this head region with its definite limited number of segments is regenerated. Consequently, the regenerated head may be attached to a quite different body region than normally. What happens then is the reorganization of the body region adjacent to the head into the normal postcephalic morphology. Among the oligochaetes the principal changes are the transformation of the intestine and the blood vessels; in tubicolous polychaetes, more striking alterations occur. In sabellids and serpulids, following a cut through the thoracic or abdominal regions, a head of three segments regenerates at the cut surface and this induces the reorganization of the adjacent old body segments into a thoracic region of typical segment number and morphology. Setae fall out, new ones appear, and the parapodia become transformed into the type characteristic of the thoracic region (Gross and Huxley, 1935, Berrill and Mees, 1936).

This reorganization of old parts to fit the regenerated head has long been known among lower invertebrates but is more obvious in annelids because of their greater morphological differentiation. The same phenomenon is seen

in coelenterates and flatworms where the head develops directly at the cut surface regardless of level, and intermediate regions must be reorganized from the old tissue. The important generalization which emerges from these data is that such reorganizations do not take place unless a head is regenerated. As phrased by Child long ago from studies on planarians, structures anterior to the level of section are not reformed unless a head or some approach to a head is first regenerated at the anterior cut surface. The only exception to this rule of which I know is one case in *Sabella* cited by Gross and Huxley (1935), where thoracic reorganization is said to have been initiated adjacent to the anterior cut surface without the occurrence of any head regeneration at that surface. One is inclined to believe there was some error of observation in this case, especially as no histological investigation was made.

It is evident that the head acts as a dominant or organization center from which some sort of influence extends posteriorly inducing the adjacent body region to transform into a normal postcephalic region typical of the species. The nature of this influence is of course debatable and various theories have been advanced. I am in accord with the conclusions of Child that the influence is transmissive in nature and the same type of explanation has been advanced by Berrill and Mees (1936) from their work on *Sabella*.

Ivanoff (1928), has attempted to explain on embryological grounds the very exact regeneration of a certain number of segments at the anterior cut surface in many annelids. It is well known that the trochophore larva often develops three segments and then pauses for a time in this three-segmented condition. Ivanoff believes these three larval segments constitute the three head segments of polychaetes like the sabellids and serpulids. In regard to polychaetes having a larger number of head segments, Ivanoff has attempted to show that the number of larval segments also corresponds; but in many of these forms,

the larva fails to pause at a definite stage so that the number of larval segments can not be determined with certainty. Chaetopterus larvae have nine larval segments, whereas the adult possesses a cephalic region of eleven segments. Since data on oligochaetes bearing on the matter were not available, Ivanoff undertook researches on the development of tubificids and lumbricids. He reached the conclusion that in these annelids also there is a definite number of larval segments, about six. Ivanhoff is thus of the opinion that when annelids regenerate anteriorly, they regenerate only the larval segments; but the facts already reviewed in this paper indicate that such an explanation, while no doubt containing some truth, can only be of rather broad application and can not be taken too specifically. According to Ivanoff the larval segments are those supplied by protonephridia and since these are evanescent, the head segments of annelids are devoid of nephridia. The transformation of abdominal into thoracic segments in the regeneration of tubicolous polychaetes is attributed by Ivanoff to the ingrowth into these segments of regenerated thoracic-type nephridia.

In the majority of annelids head regeneration is very definitely related to body level. In those forms which regenerate a head readily at almost any level and size of piece, such as naids and some syllids, it may be difficult to perceive any relation between antero-posterior level and reformation of the head. Generally, however, such relation is obvious. It may express itself merely as rate, in that the regeneration of the head is slower, the more posterior the level. More often a qualitative difference appears, especially when short pieces are employed. Thus in *Lumbriculus inconstans*, the typical head of seven segments is regenerated at any level in long pieces; but when short pieces are taken only the more anterior levels regenerate a normal head. More posteriorly the regenerated heads tend to be hypomeric with reduced number of segments and reduced prostomium and brain, and may take on a tail-like structure (Hy-

man, 1916). As already mentioned, there are many annelids in which the capacity for head regeneration ceases at some more or less definite level of the body axis. In lumbricid earthworms, the number of head segments regenerated declines as the level of cut recedes posteriorly and head regeneration ceases at about the fifteenth to twentieth segments. Similar conditions exist in the lumbriculid *Rhynchelmis* (Janda, 1918), the polychaete *Clymenella* (Sayles, 1936) and many syllids. *Chaetopterus* is remarkable in the abruptness with which head regeneration ceases; at the fourteenth segment, a complete head of eleven segments is regenerated, and at the fifteenth segment and beyond, no head regeneration whatever occurs (Berrill, 1928). Syllids may show a very abrupt decline in anterior regeneration at the level where the head of the sexual stolon forms. In some annelids, tails regenerate from anterior surfaces at levels posterior to those capable of regenerating heads.

A specific explanation for these facts is wanting. It is very difficult to see why a head can regenerate at, say, the tenth segment, and not at the fifteenth, when these two regions appear to be morphologically identical. Since any of these levels is capable of regenerating long tails of many segments, it can not be said that some element necessary for regeneration declines in numbers along the axis. It appears rather that some physiological condition associated with antero-posterior axiation is involved, as postulated by Child. It was intimated above that the regeneration of the head is a unique and more or less independent process which after it has gotten under way influences and controls the morphology of adjacent regions within a certain distance. Subordinate parts as those posterior to the level of section regenerate in correlation with antecedent parts, but for the initiation of the dominant, independent part, the head, certain physiological conditions must be established, primarily the breaking away from correlative influences. There

can be little doubt that the nervous system plays an important role in this escape from correlative influences. The nervous system is already organized for transmission in an antero-posterior direction. Because of this, an anterior cut surface is not subject to any extent to impulses from behind and can readily achieve independence, whereas a posterior cut surface is from the start subordinate to anterior regions by means of the nervous system. The assumption that the antero-posterior organization of the nervous system is best developed in anterior levels and becomes less and less effective as one proceeds posteriorly along the axis through the formation of postero-anterior paths would explain the general rule of antero-posterior decline in ability to regenerate a head.

A variety of data supports the belief that the central nervous system is of great importance in anterior regeneration. The better differentiation of the nervous system in annelids has made this group the favorite material for experiments of this kind. A number of workers have attempted to determine the role of the nerve cord in head regeneration in lumbricid earthworms by removing the cord from the segments adjacent to the cut surface. Recent experiments of this sort (Siegmund, 1928, Avel, 1930, 1932a, Kropp, 1933, Crowell, 1937, and Bailey, 1939) have confirmed earlier work that the head and brain may regenerate in the absence of the nerve cord at the cut surface. However, it seems to me that the emphasis has been wrongly placed in experiments of this type. In the majority of cases, a head fails to regenerate and, in general, head regeneration is much retarded and often imperfect in the absence of the main nerve cord. Hence the experiments really demonstrate the importance of the nervous system in anterior regeneration, in other words, the difficulty encountered by an anterior cut surface in establishing dominance without the assistance of the already established antero-posterior organization of the nervous system. Other types of experi-

ments testify to the morphogenetic action of the nerve cord. Thus Avel (1930) has shown that a head regenerates at the cut end of the ventral nerve cord when led to the body surface. A head regenerates at the anterior suture of a graft of ventral body wall containing nerve cord into the dorsal wall; if the nerve cord is absent, the graft simply heals in. Excision of a small piece of nerve cord of *Lumbriculus* induces the regeneration of a head in anterior levels, a tail in posterior levels (Haffner, 1928, 1931, Colley, 1931, Zhinkin, 1937), or of two outgrowths, an anterior tail related to the part of the worm in front of the wound, and a posterior head related to the part of the worm behind the wound. Cuts through the ventral cord of polychaetes may also induce head regeneration in appropriate body levels (Sabella, Gross and Huxley, 1935, Myxicola, Okada, 1932).

In lumbricid earthworms the ventral body wall also has a morphogenetic influence in head regeneration (Avel, 1932b, 1937). If dorsal integument at anterior levels is replaced by ventral integument including the nerve cord, two heads are regenerated, oppositely oriented. If ventral integument is replaced by dorsal integument, leaving the ventral cord in place, head regeneration is slow and feeble and often inhibited. If the ventral body wall of segments twenty-seven to thirty-seven (which are incapable of head regeneration) is replaced by a strip of ventral body wall from segments four to eleven, a normal head is regenerated following a cut through the grafted region; but if the experiment is repeated using dorsal body wall, a reduced head is regenerated in about 25 per cent. of cases. Various experiments indicate that the intestine has no morphogenetic action in anterior regeneration.

As already indicated, posterior regeneration is of less interest than anterior regeneration, but some points merit mention. Posterior regeneration is practically identical in macroscopic and microscopic details with normal growth. As we all know, chaetopod annelids

grow continuously at their rear ends by means of a growth zone in front of the anal segment; in this zone new segments are laid down in an antero-posterior direction. Only a very few oligochaetes and polychaetes have in the adult stage a definite number of body segments beyond which no increase occurs. Examples are *Clymenella* with twenty-two segments (Sayles, 1932) and *Dodecaceria* (Dehorne, 1924, 1932) with about twenty-five. It is stated that these polychaetes in posterior regeneration replace exactly the number removed. The polychaete *Polyopthalamus* (Stolte, 1929) is highly peculiar in that it does not regenerate posteriorly at all but merely heals the wound. Apart from these few exceptions and ectoparasites such as the Branchiobdellidae, any oligochaete and polychaete regenerates an indefinite number of segments from almost any posterior cut surface. As a rule the intestine fuses with the body wall forming a new anus, a growth zone is established in front of the anus, and new segments then arise as in normal growth. Such experiments as have been performed upon this point show that the length of posterior regenerate is greatest in anterior levels and declines posteriorly. This fact is in line with the suggestion that regeneration is related to the degree of antero-posterior organization, especially in the nervous system. In anterior levels where this organization is greatest the correlative influences acting upon the outgrowing tail will be strongest. In line with this explanation is also the fact that in some annelids, as earthworms and *Lumbriculus*, a tail may regenerate in place of a head at a cut anterior surface in posterior body levels. Wherever because of a decline in general antero-posterior integration, an anterior regenerate is unable to escape from adjacent correlative influences, it will develop under control of such influences into a subordinate part, or tail. The importance of the main integrative system, the nervous system, for posterior regeneration has been shown in several cases. Thus in the polychaete *Nereis* (Holmes, 1931) and the oligochaete *Rhynchelmis* (Zhinkin, 1936),

normal posterior regeneration does not occur if the nerve cord is removed in the segments antecedent to the wound. In *Rhynchelmis* following removal of a strip of cord, a tail may grow out from the cut posterior end of the cord.

It is generally true among oligochaetes and polychaetes that the cephalic region is unable to regenerate a tail unless the piece also includes a minimal number of trunk or thoracic segments. In tubicolous polychaetes often the cephalic plus the thoracic region is incapable of posterior regeneration or forms a tail in only a small percentage of cases. The exact number of body segments which must be left attached to the cephalic region before tail regeneration will take place has been determined for a number of annelids but no particular light has been thrown thereby on the matter. To say that the cephalic region is too differentiated to undergo the de-differentiation necessary for regeneration processes is only restating the fact in other words. Besides, we know that any part of this cephalic region will replace its anterior segments if these are cut away so that it is actually capable of regeneration processes. Some authors have attributed the phenomenon to the lack of nephridia in the cephalic region or the lack of other elements; but it has not been proved that the exact number of body segments which must be left attached to the head region before tail regeneration can occur exactly corresponds to the level where nephridia or other special parts or cell types begin.

It remains to consider the matter of the histology of the regeneration process. Annelids have been widely employed as material for the study of this question. In the early days, investigators were chiefly concerned with the germ-layer question, whether in regeneration new parts arose from the same germ layers as in embryonic development. Nowadays this question has faded from view and it appears further that the findings of the older workers were often in error. Consequently, only the later work is here considered.

In the study of regeneration processes in planarians and annelids, it is usually found that an indifferent type

of cell, variously termed formative cell, regeneration cell, or neoblast, plays an important role. In planarians, Curtis and his associates (1934) have suggested that regenerative power is directly dependent on the occurrence and number of these cells, without denying that other factors may also be concerned. They have shown that in genera of the Planariidae, such as *Dugesia* (= *Euplanaria*) with high regenerative power, the formative cells are abundant throughout the parenchyma, whereas in genera of the Dendrocoelidae, such as *Procotyla*, with poor regenerative ability, the formative cells are scanty. However, their own data show that formative cells do exist throughout the body length of *Procotyla*, including regions incapable of head regeneration, and that these cells take part in head regeneration as in other planarians. Attention may also be called to the fact that *Procotyla* annually with the approach of cold weather regenerates its gonads and a complex copulatory apparatus from these same formative cells. Clearly, *Procotyla* has an adequate stock of formative cells and these can regenerate a copulatory apparatus in a region of the body which is not able to regenerate a head. Again, it is the regeneration of the head which gives difficulty, and I believe this depends on other factors than the presence or absence of a certain kind of cell.

Among annelids, the data as a whole fail to support the formative cell hypothesis. In the various genera which have been investigated the histology of regeneration does not follow any one plan. Annelids in general possess a stock of indifferent cells, usually called neoblasts, which normally are found apparently inactive, on the septa and in strands along the nerve cord and muscle bands. The story of their role in regeneration differs with different genera. In posterior regeneration in *Chaetopterus*, Faulkner (1932) reported that the neoblasts multiply, migrate into the blastema, and differentiate into all the new parts of the regenerate including the epidermis. She gave a similar account of the formation of the new zooid in fission in the serpulid *Filograna* (1930). Probst

(1931) agreed with this account for *Aricia*, that in posterior regeneration practically all new parts originate from neoblasts, but in *Owenia* (1932) found an entirely different state of affairs. This polychaete has no supply of neoblasts; the new epidermis and gut epithelium come from the old ones, respectively, and all mesodermal parts arise from de-differentiated muscle. Most of the available accounts, however, especially in oligochaetes, report that the new epidermis, nervous system and other epidermal structures come from the old epidermis; the new gut epithelium originates from the old one, and mesodermal structures are formed from neoblasts.

From these varying accounts certain facts pertinent to the neoblast theory emerge. Thus, *Aricia* has a plentiful supply of neoblasts which form practically the whole of the regenerate, but is quite incapable of regenerating a head at any level. Faulkner's histological study of regenerating tails in *Chaetopterus* was done on cuts through the abdominal region; this region also can not regenerate a head, although as Faulkner showed, it is well supplied with neoblasts active in regeneration. The polychaete *Polyophthalmus* can not regenerate either a head or a tail at any level; yet according to Stolte (1929) has mesoderm cells of the neoblast type which perform the work of wound healing, so that, as Faulkner remarks, there is no histological reason for the lack of regenerative power of this animal. Although Turner (1935) found neoblasts participating in the anterior regeneration of the American species *Lumbriculus inconstans*, Ivanoff (1903) and Zhinkin (1932) agree that neoblasts are absent in head regeneration in the European *L. variegatus*. Kreckler (1923) and Stone (1933) agree that neoblasts are not concerned in anterior regeneration in tubificids. In all of these oligochaetes, neoblasts are present in all trunk levels and give rise to mesodermal structures in posterior regeneration. From these facts it is evident that in annelids ability to regenerate a head is not correlated with the presence or abundance of neoblasts.

Recent work indicates that neoblasts may not be a defi-

nite cell type. Meewis (1934) found in *Chaetogaster* that each tissue has a supply of replacement cells, called basal cells by American workers, which reform that particular tissue in regeneration. The neoblasts are simply a stage in the alterations undergone by these replacement cells when they become active in regeneration. Similar facts have been reported for other oligochaetes by Kreckler and Turner. Apparently then, the cells that accomplish regeneration are not undifferentiated embryonic cells but are already to some extent differentiated tissue elements and their transformation into neoblasts is a process of dedifferentiation.

It may be concluded that the central problem in annelid regeneration is the formation of the head since posterior regeneration is identical with normal growth. The regeneration of the head does not appear to be related to general morphological construction or to the presence or absence of particular histological elements, but rather depends on physiological conditions associated with antero-posterior axiation.

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CONTRIBUTIONS TO THE PROBLEM OF REGENERATION IN PROTOZOA¹

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I. INTRODUCTION

A. Material

THE Protozoa occupy a paradoxical position in the animal kingdom. These micro-organisms contain the solutions to fundamental problems of structure and organization within a single plasma membrane; yet relatively few significant experiments have been directed to the solution of these problems. The explanation does not lie wholly in the small size of these organisms, for some measure up to 2000–5000 μ ; they can be cut precisely, and some have even responded to transplantation experiments. A more important reason seems to be the persisting conception of the *cellularity* of the Protozoa, with attendant emphasis on research in their reactions as cells rather than as organisms.

As Perty (1852) early pointed out, to be followed more completely by Dobell (1911), the body of a protozoön can not be compared to a metazoan cell, for one would thus be comparing a whole (the protozoan organism) to a part of the whole (the metazoan cell). From the point of view of the present discussion it would seem more promising to treat the Protozoa as organisms, subject to the same general factors of growth and individuation, of reconstitution and regulation, which operate in their differently organized metazoan descendants. Then the experimental aspects of protozoan development and reconstitution may be presented in the same general manner as has been done so successfully for the Metazoa.

¹ This report has been adapted for the purposes of the present symposium from a review now in press for the *Quarterly Review of Biology*. A complete bibliography covering protozoan regeneration accompanies the review.

B. Approach to the Problem

The problem of regeneration in Protozoa has been dealt with in two general ways. Most work up to the present time has been concerned simply with tracing the extent of regenerative capacity in the various groups. This approach is primarily descriptive, and can not give much insight into the nature of causal factors in differentiation.

A more fruitful approach involves experimental attempts to discover the mechanisms at work in regeneration. It is relatively simple to separate nuclear and cytoplasmic elements by cutting experiments, and thus to trace the relative roles of each of these important spheres of influence during reconstitution. No general unanimity exists regarding the nature of the controlling mechanisms. Some have stressed the role of cytoplasmic components in regeneration. Other investigators have placed emphasis on the concept of formative substances elaborated under the guidance of the nuclear apparatus. Relations of physiological dominance and subordination along major axes have been demonstrated in some forms. The relative importance of each of these lines of research remains to be seen.

Whatever may be the nature of the mechanisms, it seems clear that they must be closely related to those operating in normal reorganization processes (*e.g.*, in fission, encystment, sexual reproduction). This follows from the fact that both in normal and experimental reorganization of a given protozoön, the actual processes of simplification and re-differentiation follow the same fundamental paths, even when they prove circuitous. This condition lends added importance to the problem of regeneration in Protozoa. If one basic mechanism operates both in normal and experimental reorganization, then regeneration also partakes logically of the nature of a developmental process and its data should prove applicable in researches on developmental physiology.

C. Scope

In the present discussion some of the more representative Protozoa will be considered in the general order of advancing complexity. Wherever possible, some of the factors underlying morphogenesis will be emphasized.

II. SELECTED STUDIES OF PROTOZOAN REGENERATION

The capacity for regeneration has been demonstrated in all the principal groups of free-living Protozoa. It seems advisable, however, to eliminate from the present review the flagellate forms (Mastigophora) and the exclusively parasitic Sporozoa. Few experiments have been performed on flagellates, and these have involved highly specialized forms not truly characteristic of the group (Noctiluca, Ceratium, Volvox, etc.). The data involving Sporozoa (only gregarines) are still inadequate, since these forms can not be cultured satisfactorily even under normal circumstances. This leaves two large groups of organisms, the pseudopodia-bearing Sarcodina and the ciliate Protozoa.

A. Sarcodina

(1) *Amebae*. Owing to their relatively simple organization, most work on these forms has resolved itself into testing the effects of enucleation on various vital processes, including regeneration. No essentially new data have been added since Gruber (1886) demonstrated that regeneration can occur in *Amoeba proteus* only in the presence of nuclear substance. Additional investigations have indicated that enucleation inhibits the assimilation of ingested food and results in a general metabolic breakdown. The qualitative unimportance of cytoplasm is indicated by the viability of as little as one eightieth of the cytosome in the presence of the nucleus (Phelps, 1926).

(2) *Shell-bearing Sarcodina*. The Testacea and Foraminifera present additional derived structures for experimentation. Verworn's work (1888) included a

comparative study of a representative of each group. Cuts on the testacean *Diffugia* never resulted in the regeneration of the shell, and this has been corroborated on numerous occasions. The foraminiferan *Polystomella* (= *Elphidium*) *crispa* reacted quite differently. Injuries to the body and shell were followed regularly by regeneration, so long as nuclear material remained in the fragment. Verworn reconciled this basic difference in reaction by pointing out differences in the normal developmental processes. Thus the testacean shell is formed in a single step at times of binary fission, while the foraminiferan shell adds to itself continuously during its lifetime. The formative mechanism seems to become irreversibly set at times of fission in the Testacea, while remaining labile and subject to experimental stimulation in the Foraminifera. The ability to regenerate seems to be a function of the normal developmental capacities in each of these groups.

(3) *Radiolaria*. These forms include some of the most highly evolved Sarcodina, exhibiting complex skeletal structures and a central capsule containing the nucleus. Verworn's investigation of the physiological significance of the nucleus (1892) led him to study *Thalassicolla nucleata*, a large peripylean type. He extended earlier work by showing that not only could isolated central capsules regenerate perfect organisms, but that homoplastic implantations of these into isolated extra-capsular cytoplasm insured viability of the latter (otherwise destined to degenerate). Additional evidence was given thereby to the nuclear role in control of viability, regeneration and regulation. This group should yield interesting results in such a field as heteroplastic transplantation.

B. *Ciliata*

The ciliate Protozoa have yielded the most extensive data bearing on regeneration. Complex locomotor and feeding organelles, a clear-cut nuclear dimorphism (macronucleus and micronucleus), and the presence of a

well-marked evolutionary series within the group have combined to permit various experimental approaches. The extensive data can be illustrated by referring to representative forms. It may be noted at the outset that the general regenerative capacity has proved excellent, so long as recognition is given to the occasional difficulty in recovering from the operative techniques.

(1) *Holotrichida*. This least specialized group of ciliates responds rather directly to merotomy,² judging from studies on fourteen genera of which *Paramecium* may be considered representative. Tartar (1939), author of the most recent and comprehensive experiments on *Paramecium*, has demonstrated that cutting results in the new-formation of organelles *in situ*, i.e., at the cut surfaces. Missing oral structures are restored rapidly as a primary response, while regulation of body shape and the remaining regeneration of cilia apparently occur secondarily and in dependence on the former process.

Calkins (1911a) showed that the normal individuation process (binary fission) in *Paramecium* could affect the regenerative process. Thus merozoa often divide in the original center of the body to give two very unequal offspring, indicating the presence of a "fission zone" which becomes irreversible. Such instances of fission seem to be definitely non-adaptive, since the "bud" which forms is rarely viable.

The dimorphic nuclear apparatus in *Paramecium* includes an oval, centrally located macronucleus and one or more small micronuclei. Lewin (1910) and Schwartz (1934) found that the micronucleus was dispensable for the actual regenerative process, although experimental amiconucleate strains were not indefinitely viable. Apparently the macronucleus of these ciliates exercises more direct control of vegetative cytoplasmic processes than does the micronucleus.

Other holotrichous ciliates (*Spathidium*, *Prorodon*,

² Merotomy—a term used to designate the process of cutting a protozoön into fragments. (Cf. the term *merozoa*, used to designate the fragments produced by merotomy.)

Dileptus, Frontonia, etc.) present no discordant data, although it is true that none has been submitted to experimentation to a comparable extent. As a group the Holotrichida permit the generalization that there is a well-developed regenerative capacity in the presence of nuclear material, although the limited viability of amicro-nucleate strains leaves uncertain the precise relative importance of the micronucleus in vegetative activities. All the available evidence indicates that regenerating organelles (cilia, mouth region, etc.) arise *in situ* at cut surfaces rather than in connection with localized reorganization centers.

(2) *Spirotrichida*. These ciliates present more complex ciliary organelles and a more highly polarized organization than the Holotrichida. Within the group there can be traced a series from forms with an adoral zone of membranelles and simple body ciliation (Heterotrichina) through some in which the body cilia are strongly reduced (Oligotrichina) to a highly organized group possessing an adoral zone of membranelles and a restricted group of derived ciliary organelles (cirri) on the ventral surface (Hypotrichina). Correlated with this trend, the regenerative response tends to become more specialized.

(a) *Heterotrichina*. *Stentor* has been subjected to more regeneration experiments than any other protozoön (23 studies). Its trumpet-shaped body bears an adoral zone around the expanded anterior end; the macronucleus is a long moniliform structure and is accompanied by many small micronuclei. Between the time of Gruber's original experiments (1885) and the latest study (Schwartz, 1935) a well-rounded picture of regenerative capacity has been constructed.

Stentor responds readily to cutting experiments. Even relatively small fragments from the middle or posterior regions of the body can reconstitute a perfectly normal individual, providing only that a portion of the nuclear apparatus is present. The regenerative process itself

follows a precise, characteristic course. It is introduced by the appearance along the left side of the body of a dense, irregularly beating mass of short cilia. From this "reorganization zone" or "anlage," there differentiates a miniature adoral zone with oral and aboral ends; subsequent migration transfers this structure to the anterior end of the merozoön where it assumes the in-rolled orientation typical of Stentor. A rapid regulation of body shape and regeneration of body cilia complete the picture of cytoplasmic reconstitution. The nuclear phase of reconstitution involves contraction of the remainder of the moniliform macronuclear chain into a "balled" stage, following which an elongation and re-segmentation restore the vegetative condition. The behavior of the micronuclei is not so clear-cut; apparently they react by tending to multiply.

It is an interesting fact that the regenerative process in Stentor simulates very closely the normal process of asexual reproduction. In both instances a daughter adoral zone arises as a localized, immature anlage that subsequently migrates to its definitive position. Furthermore, the macronucleus undergoes an identical "balling" and "re-segmentation," and the micronuclei divide mitotically, in each instance.

The problem of the relative importance of the macronucleus and micronucleus for regeneration in Stentor has been subjected to experimental study (Schwartz, 1935). This German investigator undertook the direct approach of producing amacronucleate and amiconucleate merozoa and testing their responses. He found that, while amacronucleate individuals invariably died without evincing any ability to carry on digestion and assimilation, amiconucleate individuals regenerated perfectly. Indeed, from the latter Schwartz was able to culture amiconucleate clones which led a normal vegetative existence for more than a year. Verification of Schwartz's data will provide the first entirely satisfactory demonstration of the guiding role of the macronucleus in regeneration and the utter dispensability of the micronucleus in vegetative life.

The genus *Licnophora* is a highly specialized ciliate whose closest affinity has been sought by some among the Peritrichida (*Uroceolaria*, etc.), and by others (more recently) among the Heterotrichina. Work by Stevens (1903) seemed to indicate a very slight regenerative capacity, with replacement of missing organelles occurring only *in situ* and in the presence of both macronucleus and micronucleus. A more recent study (Balamuth, 1939) has disclosed, on the contrary, a broad regenerative capacity requiring the presence only of macronuclear material. More importantly, reconstitution of adoral membranelles always involves the formation and subsequent migration of a localized anlage, as in *Stentor*. This behavior entirely supports the view that *Licnophora* is a modified heterotrichous ciliate. In all, experiments have involved eight genera of Heterotrichina, and in none has any contradiction been leveled against the newer findings for *Stentor* and *Licnophora*.

(b) *Hypotrichina*. The hypotrichous ciliates comprise the end-group of the complex Spirotrichida. All traces of the primitive, uniform ciliation have disappeared, leaving only composite ciliary organelles (membranelles and cirri) localized on the ventral surface and a highly polarized body plan.

The genus *Stylonychia* has been the subject of several experimental investigations, of which those of Dembowska (1925; 1938) are most important. A remarkable regenerative capacity has been demonstrated; this involves the regeneration of an entirely new set of ciliary organelles and cytostome whenever merotomy removes one or more of these structures. The mechanics of replacement includes the formation of localized anlagen for the various groupings of organelles, followed by their gradual migration to their definitive positions *pari passu* with the resorption of the remnants of the old set of structures. This process resembles the mode of new-formation of organelles during fission, including even the site of the anlagen.

It becomes apparent that with advancing structural complexity in ciliates, reorganization becomes progressively more integrated. In the highest forms normal differentiation is clearly divisible into a primary morphogenetic response (formation of anlagen) and a secondary response that involves migration of organelles and general regulation. It is significant that the regenerative response follows this same course, even when it proves as uneconomical of materials as it often does in the Spirotrichida. This behavior becomes understandable if we postulate that there is only one mechanism whereby protozoan structures can be replaced; whenever this mechanism is set into operation it proceeds to completion.

No data are available for regenerating *Stylonchidia* concerning the relative importance of the macronucleus and micronucleus. However, the nuclear apparatus clearly responds to merotomy by multiplying to restore the original pattern.

Study of certain hypotrichs has disclosed an apparent difference in regenerative capacity between "younger" and "older" individuals (age being based on the time interval between successive onsets of fission). Calkins' study of *Uronychia* (1911*b*) was the first in this connection. Briefly, he showed that with advancing age there was a markedly better regenerative capacity. Thus young individuals exhibited an irregular response and failed to complete reconstitution in the absence of both types of nuclei. Older individuals, on the other hand, regenerated consistently better and required the presence only of the macronucleus.

Apparently some gradual change occurs in the cytosome between periods of fission. The nature of this altered physiological condition should prove most important in analyzing the process of regeneration. Some light has been thrown on one phase of this problem by a recent study of two Russian investigators, Bauer and Granowskaja (1934*a* and *b*). Working with two hypotrichs, *Gastrostyla* and *Oxytricha*, they correlated the much higher

incidence of regeneration in older individuals with heightened respiration and macronuclear reorganization. Young experimentals reacted to merotomy by lowered respiration and macronuclear inactivity. This finding, if corroborated, may aid in explaining Calkins' results. The general relation between metabolic activity and the onset of the regenerative mechanism should be subjected to a critical study.

3. *Peritrichida*. This group is an offshoot from holotrichous forms and is distinguished by a characteristic circular arrangement of peristomal cilia and a general reduction of body ciliation. Little attention has been devoted to regeneration in the peritrichous ciliates, but at least one recent investigation is important in this connection. Summers (1938*a* and *b*) used a colonial form, *Zoothamnium alternans*, in which the component zooids are arranged in a precise frond-like pattern. He discovered the existence of a well-developed regulatory mechanism in the growth and differentiation of colonies. Thus the removal of the dominant apical member of a colony resulted in the assumption of dominance by some dependent sub-terminal zooid, and the colony proceeded to differentiate under this new leadership. Various experimental series confirmed this relation of a normally occurring dominance-subordination that apparently acts like the more widely known cases among Metazoa. Summers' data suggested also that younger colonial individuals are less rigidly determined than older ones, since the former exhibited more frequent regulatory responses.

In protozoological literature there are numerous references to the importance of physiological dominance and subordination during regeneration. It is well known, for example, that in such forms as *Stentor* and *Stylonychia* the presence of a remnant of the old adoral zone tends to inhibit the onset of regeneration of a new one. The general reaction obeys the expression, that the latent time before the onset of regeneration varies inversely as the degree of injury to the adoral zone. These and similar

observations have been interpreted as evidence of subordinating effects exerted by dominant regions. In this connection it is interesting to note that Child (1914) and later workers have demonstrated physiological gradients of activity along major axes in Protozoa. These are apparently analogous to those found frequently in Metazoa. Their importance has not been investigated in the Protozoa.

III. QUESTION OF THE MECHANISM UNDERLYING REGENERATION

As indicated at the outset, there has been no clear-cut demonstration of the causal mechanism involved in protozoan regeneration. Various stimulating suggestions have been made, and by examining some of the supporting data the present position may be briefly summarized.

In the first place, the processes of normal and experimental replacement of organelles seem to follow the same fundamental plan. The extent of regeneration is limited, in general, by the normal developmental potentialities (*e.g.*, see Testacea *vs.* Foraminifera). In each instance the fundamental problem is the preservation of species-identity, and all available evidence suggests a common mechanism in effecting the reorganization of nucleus and cytosome.

Control of the formative stimulus should be traceable either to the nuclear apparatus or to the cytosome, or possibly to some definite interaction between the two. The data collected thus far do not support the view of Loeb (1899) and others, which assigns the regenerative impulse entirely to the cytoplasm and regards the nucleus to be purely of subordinate importance.

We have seen that protozoan bodies contain physiologically dominant regions. It is necessary to test the possibility that the primary factor in both normal and experimental reorganization involves differences in metabolic activity. No physiological experiments of this kind have been conducted as yet.

The nuclear apparatus undeniably plays an important role during regeneration. Enucleate fragments never initiate regenerative processes, even though they may live and move for several days. Morphological evidence demonstrates that the nucleus (macronucleus of ciliates) undergoes complex form-changes in regenerating merozoa, and these usually begin before the onset of cytoplasmic reorganization. The extrusion of chromatin material into the cytoplasm has been frequently reported during reorganization, although this may have, and indeed need have, no connection with the transfer of specific substances. Chemical substances often remain unknown except for the demonstrable effects they produce.

The most convincing evidence supporting the importance of the protozoan nucleus in regeneration has come from the experiments on ciliates. It has been shown fairly conclusively in some of these forms that not only is nuclear material essential in regeneration, but more specifically the *macronucleus* is the controlling factor. Moreover, its influence extends to digestive and anabolic functioning, to judge from various investigations (*e.g.*, cessation of anabolic processes in enucleate merozoa), and therefore it seems to be the seat of more than one kind of secretory activity.

In this connection, finally, a recent study on an unicellular plant (the alga, *Acetabularia*) has pointed the way to a possible mechanism governing regeneration. Working with this extremely favorable material, Hämmerling (1934*a* and *b*) has shown that, as in the Protozoa, the presence of the nucleus is essential for regeneration. The method of "biological assay" of fragments enabled him to demonstrate a clear-cut gradient of morphogenetic impulse arising at the nucleus and proceeding along the heteropolar axis to the sites of new-formation. Perhaps the most significant experiment involved heteroplastic transplantation between two related species. The remarkable result was that the particular species characteristics differentiated in each instance according to the source of

the nuclei rather than the cytoplasmic mass in which the nuclei acted. According to Hämmerling, all the evidence supports the view that the effects are due to differences in concentration of formative substances, originating in the nucleus and acting at sites of morphogenesis. The orderly migration of substances may well be due to the influence of physiological gradients acting to set up local differences in accumulation.

Needless to state, it would be premature to claim the existence of such a mechanism in Protozoa. It is interesting, however, that as far as they go the observed facts seem to fit in with the mechanism suggested above. In any event, future investigations must take account of some of these unexploited lines of research, and to do so will involve greater emphasis upon the *Protozoa-as-organisms*.

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ADDITIONAL IMPLICATIONS OF REPTILIAN SENSITIVITY TO HIGH TEMPERATURES

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DURING the past year the writer has conducted extensive but as yet uncompleted investigations into the thermal responses of the supposedly heat-loving reptiles of southern California deserts. These have clearly indicated that far from being actually thermophilous animals, in the sense of favoring high temperatures, the reptiles conform to the usual thermal limitations of the metazoan protoplasm. This fact has led to a brief exposition of an hypothesis regarding heat as a factor in the history of terrestrial poikilotherms (Cowles, 1939). Subsequent consideration of the part which high temperatures may have played in the history of terrestrial vertebrates has led to the conclusion that additional discussion and elaboration of this subject may prove of interest.

TEMPERATURE AND THE LIVING REPTILE

The optimum for most of the strictly diurnal species of lizards ranges from 37° C. (98.6° F.) to approximately 38° C. (100.4° F.). Species investigated in the field so far are, the chuckawalla, *Sauromalus obesus*; the desert iguana, *Dipsosaurus d. dorsalis*; the leopard lizard, *Crotaphytus wislizenii*; the short-nosed leopard lizard, *Crotaphytus silus*; the fringe-footed lizard, *Uma notata*; and the flat-tailed horned lizard, *Phrynosoma platyrhinos*. Optimum temperatures for the snakes lie between 31.6° C. and 33° C. for the nocturnal species such as the sidewinder, *Crotalus cerastes*, and the diamond-back rattlesnake, *Crotalus atrox*.

While Mosauer (1936) determined the approximate lethal temperatures for three lizards and one snake, the writer has found that the putative lethal temperatures

may vary widely depending on the criteria used in determining death. Due to the fact that respiration may cease several minutes prior to the termination of heart-beat, the usual criteria fail to give reliable results. There is a sharp increase in temperature the moment the cooling effect of respiration is lost, and from numerous observations it seems probable that the erratic breathing which takes place just prior to death may also influence the so-called lethal temperature. For these reasons it has been considered advisable to seek other criteria for the determination of thermal relationships at high temperatures, and investigations have consisted for the most part in a compilation of records of the temperatures at which the power of coordinated locomotion is lost.

When exposed to direct sunshine and high temperatures that prevail in spring and summer, snakes and lizards rapidly lose their capacity for well-coordinated locomotion, a situation which would normally bring about their immediate death under natural conditions. Using this point as the criterion for the practical lethal point, the method has the experimental advantage that rapid removal to a cool place will restore the animals to apparently normal conditions, thus affording an automatic check on accuracy, and a repetition of the experiment will produce almost identical results if not made too soon after recovery. For ecological purposes it may be assumed that this point, which has been termed the critical maximum, is the practical equivalent for the lethal temperature. When obtained by this method, field observations indicate an average critical maximum ranging close to 45° C. for the following diurnal lizards: the desert iguana, *Dipsosaurus d. dorsalis*; the chuckawalla, *Sauromalus obesus*; and the horned lizard, *Phrynosoma platyrhinos*, while the nocturnal snakes display a much greater sensitivity and are affected at temperatures ranging from 35.3° C. in *Sonora occipitalis* to 37.6° C. in *Crotalus cerastes*.

One of the most significant characteristics displayed

by both groups has been the considerable discomfort displayed at 3° C. above the optimum. Observations conducted over a period of three months during the spring of 1940 have verified these observations and also proved conclusively that the maximum temperatures which these lizards will voluntarily tolerate, even momentarily, lie at most only four degrees above the optimum. For any species so far maintained under observation, discomfort is so acute at temperatures between three and four degrees above the optimum that it is clear that prolonged exposures at these temperatures would inevitably result in serious damage.

Although serious discomfort and damage may result from exposures of three degrees above normal (optimum), these lizards submit freely to a lowering of the temperature to as much as 9° C. below their optimum. A further drop of 1° C., however, forces them to take refuge underground, but the capacity for locomotion apparently is not impaired seriously until a point is reached some twenty degrees below the optimum. It is also notable that while in hibernation they frequently sustain much lower temperatures. Even in midsummer, snakes and lizards may be maintained under refrigeration until their body temperatures drop to 1° C.-2° C. without suffering any apparent harm.

The remarkable degree of tolerance to cold exhibited by lizards and snakes from one of the hottest localities known and their more startling inability to withstand high bodily temperatures furnishes an intensely interesting study in adaptations of behavior. The physiological failure to cope with extremes of high temperatures has forced upon these animals a versatility in coping with the exigencies of hostile temperatures which is worthy of much study. Since it is possible that these forms may broadly represent prototypal thermal capacities, they have provided much interesting speculation on climatic conditions and the thermal ecology of the stem reptiles as well as the archosaurs.

HYPOTHETICAL IMPLICATIONS OF THE PAST IN TERMS
OF THE PRESENT

That there is no infallible method of determining the temperature limitations of the archosaurs and their prototypes is obvious, but lacking any better criterion than that which can be found in the living forms of to-day, an imaginative venture into the past has been made on this basis in the hope that the following purely hypothetical proposals will provide interesting alternates to accepted ideas.

If the heat resistant capacity of the archosaurs and their prototypes can be accepted as resembling even our most thermophilous reptiles of to-day, it appears probable that they could have been no better able to cope with high temperatures than are modern desert lizards since these are species which have crowded the thermal ceiling of physiological tolerance. It is significant that the stem reptiles survived the coldest intervals of glaciation during Permian times and that modern types retain what appears to be a primitive capacity to withstand long periods of low temperatures without harm, even to remaining active above the relatively low temperatures of 27° C.-28° C. That some of the prehistoric reptiles were endowed with a similar capacity to maintain life through a wide range of temperatures seems reasonable from a consideration of the association of fossil plants and reptilian fossil remains, but it is highly improbable that any ancestral reptiles possessed a capacity to endure temperatures higher than those which are sustained by modern forms, a reasonably safe statement since the upper limit for the most heat-tolerant metazoans is supposed to be reached at 45° C.

Extant thermophilous forms may be considered as eurythermal types with an activity range of approximately 10° C., but their upward crowding of the optimum to a point just below lethal temperatures would place them in the stenothermal group so far as tolerance of any increase is concerned. The application of Leibig's

law here, "The factor for which a species has the narrowest range of adaptability limits its existence," seems to indicate conclusively that as between falling temperatures as a reason for the disappearance of the archosaurs or an increase above the optimum, the latter factor must be given the greatest consideration.

Without too close an analysis and with full recognition of exceptions, terrestrial vertebrates may be grouped roughly in a thermal sequence representing environmental or body temperature preferences. This can be accompanied by another generalization which will, however, bear further investigation, that the more primitive forms of each group require the lower temperatures. Some exceptions exist but, in general, the series ranges as follows:

Most specialized birds	Highest temperatures	
Less specialized birds	Lower temperatures	
Most specialized mammals	Highest temperatures	
Less specialized mammals	Lower temperatures	
Most specialized reptiles	Highest temperatures	} Exclusive of the snakes
Less specialized reptiles	Lower temperatures	
Most specialized amphibia	Higher temperatures	
Less specialized amphibia	Lower temperatures	

Some familiar exceptions to this generalization may be recalled and a particularly obvious discrepancy may be found in the desert areas where the diurnal racers, although not considered as being specialized, are apparently the most heat-tolerant snakes. But while snakes are the most highly specialized of reptiles, their specialization has been such as to allow them to escape from the temperature fluctuations to which the less secretive, limbed forms are subjected and exceptions may be expected.

Investigations into thermal tolerance which are now being conducted show clearly that the snakes are incapable of sustained activity at either such low or such high temperatures as the lizards, and that, therefore, they should be relegated to a special category, possibly illustrative of the primitive, unaltered thermal requirements of ancestral types, probably perpetuated through early

adoption of nocturnality or a subterranean existence and consequent escape from the vicissitudes attendant on more exposed forms. In this connection Dr. G. L. Walls has kindly allowed the presentation here of some recently noted optical phenomena which provide independent substantiation of this possibility. His observations follow:

There is good evidence, from comparative ophthalmology, for believing that the ancient amphibians were strongly diurnal. On purely logical grounds, it is reasonable to suppose that the heavy-bodied Stegocephali would have sought the sunlight. Lacking enemies on land, they had no incentive to adopt nocturnality for purposes of concealment, and hence no need to forego those advantages of diurnality which accrue to a poikilotherm—the facilitation of locomotor activities and mental processes through the acceleration of metabolism.

The eyes of modern amphibians exhibit certain features which reveal that the diurnal forms—the frogs—have had uninterrupted diurnal ancestry since the amphibia arose from their piscine forebears. The most important of these features is the colored oil-droplet characteristic of the retinal cone visual cells. Associated firmly with diurnality wherever it is found among vertebrates, this cytological feature, once discarded, can never be regained. This is attested by the substitution for it of other color-filtering devices in diurnal snakes, diurnal geckoes, diurnal squirrels, primates, and insectivores—all of which lack oil-droplets and whose immediate ancestors were nocturnal, it being possible in every case to be certain that *their* ancestors, in turn, were diurnal and did possess colored oil-droplets.

The existence of colored oil-droplets in modern frogs is thus proof positive (to the satisfaction of a comparative ophthalmologist) that the Stegocephali, standing at the bottle-neck of amphibian phylogeny, were diurnal animals. They were probably more strongly so than the frogs, which are too small to dare tempt their many enemies and their high surface-volume ratio by exposing themselves too completely to the invigorating, but dessicating, action of the sun.

There are indications from the histogenesis of the amphibian retina that what capacity the frogs have for seeing in dim light was gained secondarily within the group; that, in other words, the frogs were once more strictly diurnal than they now are, which holds of course for the toads as well. The points involved are too complex, however, for lucid treatment in a brief letter.

If it were possible to do so, it would be exceedingly interesting to insert in the phylogenetic thermal arrangement the temperature preferences of the reptilian types from cotylosaurs to the dinosaurs, especially since there is reason to believe that the most logical position for the insertion should lie between that of the amphibia and the living thermophilous desert reptiles.

Although isolated species offer no safe criterion as to temperature relations because of possible specific modifications brought about by a response to specialized environment, it is none the less interesting to note the habitat of the nocturnal *Sphenodon* in a cool and damp environment. That the climatic factor may have been fully as important an item in survival as insular insolation is supported by the universal dependence of the crocodilians and most chelonians on an aquatic retreat where they can escape excessive heat.

With the limited information available, the association of thermal tolerance and phylogenetic arrangement can not be carried further than that given here, possibly due to the fact that even in the lower groups exceptional species have been able to take advantage of the acceleration of chemical processes at higher temperatures. The exigencies of adaptation for habitat and ecological niches, cause further confusion, but the close thermal relationship of vertebrate classes and orders strongly suggest a progressive adaptation to, and utilization of, higher temperatures. That all major groups have not been able to do so, suggests the possibility of a retention of phylogenetic limitations which may have been imposed by the environmental conditions pertaining during the origination of the group. It would appear logical to assume that environmental conditions existing at the time of development, and subsequently, might have left their imprint on the protoplasmic tolerance and so have provided us with some clues as to climatic conditions of the past and their influence on existing forms.

Although the evidence is by no means conclusive, it can readily be believed that in addition to alternating cold and warm cycles there may have been periods of excessive and continuous heat which have given rise to those forms which are now capable of taking advantage of the chemical acceleration which would accompany high bodily temperatures of around 37° C. While it is ordinarily thought that the tropical climate as now known may have

provided the requisite conditions, it appears probable that the tropics may actually be too cool. For instance, at Barro Colorado the mean annual temperature fluctuates between 26.7° C.-28.4° C. (80° F.-83° F.); in other words, approximates or even falls below the minimum temperatures to which the diurnal, heat-loving lizards will voluntarily submit, and furthermore falls roughly 10° C. below the optimum of these animals, or equally significant, 9° C. or more below that of the average homoiotherms.

Although the optimum, *i.e.*, self-selected body temperatures of tropical animals is not known, it is probable that the basking proclivities of many of the tropical reptiles represents an environmental thermal deficiency and an effort to elevate the body temperature above that of their air or water environment through a resort to insolation, and that only by this means can they achieve the required optimum velocities of chemical reaction.

TERMINOLOGY

In the light of observations and experiments on desert reptiles and the resultant conclusions which are presented here, it becomes clear that more descriptive terms should be employed for the sake of accuracy and in order to clarify the nature of the poikilotherms as a group. They fall naturally into two categories as to methods of achieving optimum temperatures. The most convenient and descriptive categories result in a division into heliotherms and thigmotherms, the former receiving their heat chiefly through basking, the latter, chiefly through contact with substances in the environment—air, soil or water. Such a division naturally leads to the collective name “ectotherm” as an adjunct of or substitute for the inadequate characterization provided by the word “poikilotherm.” The fallibility of the term is obvious since it is well known that many fish, and probably reptiles as well, live at a more constant temperature than many so-called homoiotherms.

The implication of the name "homoiotherms," which suggests the immutability of normal temperatures, is belied by many birds and mammals, and furthermore, both as a more suitable and logical contrast to the ectotherms, it is suggested that the warm blooded organisms may be conveniently referred to as endotherms. The employment of these categories is valuable not only in the study of modern forms but is also useful when applied to the theoretical conditions which may have existed in the past. This nomenclature is particularly helpful in visualizing and analyzing the possible conditions under which prehistoric organisms may have existed, or as a result of which they may have given rise to their successors.

Although the ectotherms are capable of producing some bodily heat, this factor apparently is of negligible importance throughout most of the environmental temperature ranges under which they normally operate, but it is realized that exceptions exist under conditions of great humidity with environmental temperatures slightly below the optimum, or where, as reported in incubation of the python, there may be a slight elevation of temperature due to a production of internal body heat.

If prehistoric vertebrates are thought of as being either heliothermic or thigmothermic, the question at once comes to mind as to the function which temperature may have played in the conditions under which the terrestrial habit originated.

It is known that ostracoderms were bottom dwellers and frequented shallow lakes or the littoral areas along these bodies of water. Therefore, the questions may be legitimately asked as to whether the habit of dwelling in shallow water may not have been a step toward heliothermy and that the subsequent emergence to land by later forms may not have been induced, in part at least, as a response to this upward step toward the achievement of advantages inherent in the greater velocities of chemical processes available at higher temperatures.

If the land habit evolved as a response to a gradual

drying up of the original habitat and the need for water, the emerging forms, theoretically, would have been obligated to face the dangers of dehydration as well as possibly excessive heat, or both, whereas if emergence were primarily due to the adoption of the shallow water-basking habit, alternating with short periods of land-basking, the transition from the aquatic to the fully terrestrial mode of life would have been a gradual and logical step, to be negotiated especially during times of high humidity and therefore with little hazard and many advantages.

An acceptance of this hypothesis requires the postulate that the climates of the Silurian, Devonian and Carboniferous were cool or temperate, thus encouraging or permitting a gradual adoption and dependence on heliothermy. Although this is apparently at variance with some interpretation of evidence obtained from the vegetation of the Carboniferous, it may be possible that plants of those "forests" require the cool, moist climates which, in general, are preferred by their modern descendants. In fact, the Bryophytes, Pteridophytes and Gymnosperms (apparently with some notable exceptions) suggest through their present concentration and distribution that they, as well as their ancestors, also may have required cool climates or habitats, whereas the more specialized as well as more recent Angiosperms have invaded much hotter as well as dryer areas.

If heliothermy was the chief stimulant or one of the important stimuli involved in bringing about the terrestrial habit, it is probable that these early forms either possessed or soon evolved the radiation-absorbing dark pigments so characteristic of their more highly specialized descendants, but the capacity for thermal control through color change, so essential to most lizards of today, may not have evolved until later times.

Although no direct evidence bearing on this aspect is available, it is known that while many fishes possess the capacity for color change, it appears to be of value largely in concealment, there being very few fishes which are given to basking.

That the snakes do not possess a marked capacity for color change may be due to the fact that at least the desert species are characterized by an appreciably narrower range of temperature tolerance, a condition which has been determined by investigation into the thermal point of inception of locomotion as compared with the optimum. This trait appears to indicate that these forms, through their adoption of a nocturnal, subterranean or secretive habitat have either conformed to more primitive physiological limitations, or that through their selection of environments which are characterized by smaller changes in temperature, they have become incapable of the greater tolerances exhibited by their less specialized relatives. It is also true that their mode of locomotion renders them particularly susceptible to the lethal effects of high environmental temperatures. Their prone position subjects them to the full effect of combined radiation from above and direct contact with the heated ground from below. From the studies conducted so far it appears probable that snakes represent not only the most specialized morphology but the least specialized thermal adjustment.

If the ancient reptiles, like their nearest modern relatives, the crocodiles, possessed the dark pigmentation so essential for the forms of to-day, but lacked the additional faculty for color change, now an important protective device against both overheating and a too rapid heating, there can be little doubt that exposure to temperatures even a few degrees above those to which they were adapted would have led to their rapid disappearance or would have necessitated habit and habitat adjustments. Even if possessed of this protective device they would have been capable only of somewhat longer exposures, but at the same time they would have been seriously affected by a persistent increase in the intensity or duration of insolation.¹

¹ Atsatt (1939) has pointed out that in *Xantusia vigilis* there is a peculiar reversal in color change responses with respect to heat stimulation. While most lizards are characteristically light when warm and dark when cold,

Although the geological record has provided glacial deposits, striae, alluvial and lacustrine deposits, etc., thereby providing direct evidence for marked lowering of temperature as well as a direct evidence for changes in precipitation, the evidence for slight temperature changes is entirely indirect and is available only through the distribution and types of fossil vegetation. Although there is ample evidence for periods of great aridity, the accompanying reduction in vegetation through which interpretations can be made often provide less obvious clues as to whether these arid and semi-arid intervals were characterized by high or low temperatures, and it is entirely possible that there may have been hot-dry and cold-dry intervals. In either case, the concomitant greater diurnal-nocturnal fluctuations would have imposed severe thermal changes on the ectothermotic organisms of those areas.

Although no direct evidence on these temperature changes is available, it is possible that a study of reptilian responses may suggest a logical hypothesis, and although it is possible that the archosaurs and their ancestors may have been characterized by temperature tolerances lying between those of to-day's urodeles and the desert reptiles, for the sake of argument they may be admitted as possessing tolerances as great as those of our most extreme forms of to-day.

That these ancient reptiles could have been eurythermal to temperatures much above 42° C. is entirely illogical. So far as known the protoplasm of all known vertebrates and most invertebrates appears to be subject

Xantusia has reversed the change and is dark at high and light at low temperatures. This apparent aberration is a logical result of a high, dry habitat and the nocturnal, secretive habits of the animal. It is probable that this *Xantusia* is never voluntarily heliothermotic and that therefore it receives its heat supply from intimate contact with the walls of its retreat, or the air above ground, i.e., by conduction rather than through radiation, and that the chief thermal occupation may be devoted to constant efforts to obtain the maximum available heat. It is highly improbable that it is ever faced with the problem of overheating.

to irreversible changes at temperatures of 45° C. for the Metazoa and possibly 50° C. for the Protozoa (Hesse, Allee and Schmidt).

In spite of the comparatively high temperature tolerance exhibited by such species as *Dipsosaurus d. dorsalis* and *Sauromalus obesus*, a few minutes' exposure to full sunshine on a hot spring day will induce a temperature of 44° C. and crippling within a few minutes' time. Such susceptibility in these species, probably among the most heat-tolerant of all known forms, indicates that even in the relatively mild condition of to-day there exists areas in which even the greatest possible physiological adjustments can not avert death as a result of insolation.

The larger reptiles, other things being equal, require longer exposures to produce the body temperatures that induce death, and both the desert iguana, and particularly the larger chuckawalla, are capable of extending their feeding activities later into the day than the smaller insectivorous species. This important extension of available feeding opportunities is partly a result of the favorable effect of their greater volume. From this standpoint it is conceivable that the tremendous size of many of the archosaurs, as well as the prevailing bipedal posture, were direct results of thermal limitation. The erect posture presents a smaller surface to insolation and removes the body from contact with the ground, while the great volume requires a longer period to heat as well as a longer period in which to cool off during the night. All of these signal developments can be readily explainable in terms of increasing heat and aridity together with the inevitable accompanying reduction in the size of xerophytic plants, and resultant decrease in available shade.

It would be expected that ectotherms subjected to increasing heat and reduced shade might seek escape in two major directions: (1) through an increase in size, which would eventually lead to a fatal thermal *cul de sac* in case of continued great increase in temperatures; or (2) through a reduction in size which would allow these

smaller forms to take refuge underground during the day-time to emerge at night.²

From recent study in the ecology of desert animals it is clear that one of the chief advantages of the subterranean mode of life results from the equable temperatures which are thus easily attainable in a super hot habitat, but even in deserts it does not provide complete escape from numbing cold. In addition to this advantage, underground retreats in hot deserts allow a selection of almost any desired temperature, these being readily available through penetration to the appropriate levels in the ground.

Small carnivorous and particularly insectivorous species of animals are provided with ample food supplies in these underground retreats, since burrows and caves are also used as refuges by all other small, therefore highly heat-susceptible ectotherms, a factor which explains the success of the almost universally adopted troglodidic habit. During periods of moderate temperatures, such as those occurring during the spring and fall of the year, the reptiles emerge from below ground, at first maintaining optimum temperatures by a constant shuttling movement to sun or shade alternating with periods of cooling in their underground retreats. During the autumn, surface activities are extended as temperatures drop from mid-summer maxima, *i.e.*, the animals become heliothermotie as soon as air temperatures fall below their optimum. Eventually all forms are once more driven to shelter by the long cold nights of approaching winter and the resultant cooling of the ground. The activities of prehistoric ectotherms may be envisaged as having been very similar under the same environmental conditions.

While fantastic, it may not be impossible that the great proliferation of armor and dorsal "fans" or "sails" (pelycosaurs and Stegosaurus, etc.) may have evolved, in part, as a device to avoid radiation of the tissues con-

² The absence of abundant sedges and grasses until Tertiary times is of great importance in this connection.

taining capillaries and other vascular therefore heat-conducting structures, from unmodified insolation. These would be of particular value over the region of head, neck and back as a protection to vital nerve centers from heat as well as enemy attack, thus serving a dual purpose.

As an alternative to the development of one or the other of these adaptations it is entirely possible that there may have been both annual north-south as well as similar major cyclic migrations. While seasonal migrations might have been possible for the more active forms, this recourse for the evasion of heat may not have been available to the larger, more cumbersome species.

The presence of palms and figs, corals and other warmth-loving organisms as far north as Alaska and Greenland during Cretaceous times seems to prove conclusively that these areas must have been tropical or subtropical in nature for part of the year, but the long nights and short days of winter must have produced conditions utterly unlike those of the equatorial tropics which are characterized by equal length of the days and nights. South of these pseudotropical areas, especially between 30°–35° north and south, the long days of summer must have produced intolerable heat, especially during periods of drought, and it is difficult to conceive of the equatorial tropics as other than intolerably hot during the short days, while remaining proportionally hot throughout the short nights.

Resort to migration to escape excessive heat may have produced a northward summer movement and a southward winter retreat, the latter presumably as a result of the long nights and by a possible contingent partial dormancy of vegetation. Since these annual seasonal movements may have been available only to the more agile species, it is conceivable that it may provide an explanation of the origin of the migratory habit which is so strongly developed in birds, but with the original north-south migration first adopted in order to escape heat. With the remission of excessive heat and a return to a

mild climate, diurnal fluctuations due to the changes in available daylight might perpetuate the movement. With a continuation of the climatic trend, cold weather would accentuate the movement once more, but throughout the entire cycle, seasonal movements would remain unaltered. It is even possible that high as well as low temperatures may explain the common transtropical movement.

The slow advance and retreat of the major nonseasonal cyclic changes would presumably have allowed a long range species diffusion (as contrasted with individual migration) to take place from the tropics northward and southward with the onset and development of excessive equatorial heat. While it is difficult to explain the extermination of archaic forms at the northerly and southerly limits of the continents where climates were presumably moderate, it is possible that the extremely long days, even though producing less intense insolation, in conjunction with the short nights, too short for effective cooling, may have been one of the factors instrumental in causing their death. This may be explained by a comparison with desert conditions, where black-bulb thermometers exposed in reptilian habitats frequently register as much as 85° C. (185° F.). Such conditions cause death in a few minutes at between 45° C.-50° C., but identical results are achieved with prolonged exposure to the more moderate early season sun and air temperatures which together produce body temperatures of between 45° C. and 50° C. Since such moderate but prolonged temperatures were by no means impossible during the Mesozoic times, no great stretch of imagination is required to visualize the ultimate destruction of ectothermotic animals. They may have been driven from equatorial regions by the high diurnal temperatures, and in retreating polarwards encountered, and may have been trapped by, less intense but greatly prolonged exposures to lethal heat. Supporting evidence for fairly high temperatures is to be found today in the geological records of the Argentine and Alaska.

Although it is difficult to explain the nearly simultane-

ous disappearance of the marine reptiles toward the close of the Mesozoic, the survival of crocodilians and chelonians indicates that some other factor than the direct effects of temperature may have been involved for aquatic ectotherms. Had these forms been oviparous there would be little difficulty in understanding their demise, but since at least some aquatic species are known to have been viviparous, it would be supposed that the young would not have been subjected to insuperable thermal hazards. As an alternate suggestion it may be supposed that some indirect effects of temperature changes may have altered the food supply, possibly either by a destruction of the prey, or by driving it to deeper, cooler water. The gill-breathing fishes could and might readily have retreated, while the air-breathing reptiles would have remained behind.

One of the most noteworthy aspects of the ectotherms as contrasted with the endotherms is the complete absence of effective insulating structures in any of the terrestrial forms, probably a condition which existed also in the ancestral prototypes. While internal insulation provided through layers of hypodermal adipose tissue is a common occurrence in the endotherms, whether endowed with surface structures or not, no such massive deposits are present in any terrestrial ectotherms. This characteristic appears to be directly connected with their dependence on outside sources for effective heating, and suggests the negligible value of such internally produced heat as is available. Apparently any attempts to conserve internally generated heat through external coverings so reduces the efficiency of the mechanism for heat absorption at moderate temperatures as to have prevented such a development. In this connection it therefore becomes essential to consider the circumstances under which the transition from ectothermy to endothermy may have come about.

Paradoxically, it seems logical to believe that the external insulating covering of birds and mammals

may have first originated as a device for protection from extremely high environmental temperatures, particularly as a defense against insolation. The total absence of any conspicuous insulating devices except color change in living reptiles, and the disadvantages which would result from either internal or external insulating devices, suggests that ancestral reptiles, birds and mammals, would have likewise suffered had these coverings been evolved under the conditions generally assumed to have been responsible for their initiation, that is, as a device to conserve internal heat in the face of a cooling environment. It is clear that external, insulating coverings would be hazardous for ectotherms since internal heat generation is ineffective, and such insulation would tend to occlude the needed heat of radiation.

If excessive heat played the part hypothecated here it is logical to assume that endothermism followed the evolution of types of ectotherms living under conditions of excessive insolation and having as one factor in their survival an effective mechanism of protection from radiation and a provision for heat occlusion. Certainly the lizards of our deserts, if endowed with either fur or feathers, would be capable of very greatly extending their hours of daylight activity during the hot season, simply through this protection from insolation. Furthermore, the low production of internal heat in these animals would enhance the value of such a covering under these conditions. Conversely, the possession of such a protection would be a severe handicap during cooler weather since it would exclude the necessary external heat. Considering the matter from another point of view, the possession of a coating of insulating substances which was capable of being erected so as to permit direct insolation of the skin during cooler weather, with a mechanism for lowering or closing up of the structures as the available outside heat dropped below that requisite to maintain the optimum, would extend enormously the length of the effective part of each day that could be profitably utilized by these animals.

While it is generally assumed that endothermism (homiothermism), developed as a response to falling temperatures as a device to substitute body-generated heat for external supplies, this development would have required the simultaneous appearance of internal heat production and control mechanisms, operating in conjunction with the external insulating characteristics. While the development of dual interlocking or interdependent characters of this type could have taken place simultaneously it seems less plausible than that feathers and fur were originally evoked as a heat-excluding device, and that with the slow recession of the heat wave, followed by cooling nights and warm days, the prototypes of the birds and mammals may have resorted to partial basking made possible through the elevation of the feathers (and fur in the mammals), a particularly notable characteristic of the birds of to-day. Such behavior, followed by use of the same structures to occlude heat during the hottest part of the day and for the retention of externally acquired heat during the night plus an increase of the heat produced from within, thereby making it possible to carry over the optimum from day to day, may eventually have produced the types of endothermotic organism with which we are familiar to-day, *i.e.*, warm blooded forms with fairly uniform temperatures, maintained through heat production and conservation which has become possible through the presence of an ectodermal insulation of feathers or fur and subcutaneous layers of insulating fat.

SUMMARY

A presentation of an hypothesis to explain the great increase in size, the bipedal posture and the sudden disappearance of the giant reptiles, with an interpretation of their death in terms of the fatal susceptibility of present-day reptiles to temperatures in excess of 45° C. and the known thermal limit of 45° C. for most metazoans. While positive geological evidence for lethal high temperatures is not available, indirect evidence suggests the

possibility of extreme heat in circum-equatorial regions, which may have been comparable in intensity to the low temperature extremes which left their evidence in glaciation. If such an explanation is acceptable, the devices of feathers and fur may have evolved originally as a protection of skin and body from excessive insolation, rather than to conserve a probably negligible internal heat. The eventual development of effective internal heat production in conjunction with the previously supplied insulation, may have come as a response to the ebbing heat waves and thus have produced the comparatively efficient homoiotherms of to-day.

It is proposed that for accuracy and clarity the term "ectotherm" with the subtypes "heliotherm" and "thigmotherm" be substituted for "poikilotherm," and that "endotherm" be substituted for "homoiotherm." When considered from the viewpoint of the organic requirements of heat, there appears to be a definite progression away from the vagaries of thigmothermism through the adoption of heliothermism, with eventual stabilization by endothermism.

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SHORTER ARTICLES AND DISCUSSION

THE MASQUERADE OF LAMARCKISM

Structure is after all the visible, highly mechanized end-stage of function, and our inability to detect the inheritance of an acquired character is probably due to the fact that its visible appearance is preceded in phylogeny by a period of many generations during which it is inherited only as a function associated with alterations of structure too subtle to be revealed by our present very crude methods of observation and experiment.—*W. M. Wheeler.*¹

No change has yet been induced in any chromosome mechanism which has any adaptive relations to the circumstances inducing it. . . . Hereditary properties always arise in advance of the uses to which they are put.—*C. D. Darlington.*²

INNUMERABLE records of critical experiments and authentic observations are available to support the general assumption that organs, individual organisms, species and biocenoses are capable of wide ranges of somatic and aggregative variability which are certainly specific responses to phases of nurture. Commensurate, excess or inadequate diet, extremes of heat or cold, moisture or aridity, exercise or lassitude, glandular substances, other and undetermined environmental factors have been shown expressly and specifically to modify the bodies or organs of animals and plants and their associations. The essence of Lamarckism is the conjecture that by some undefined means, or parallel and specific induction of the germ-plasm, perpetuation of these induced adaptive modifications is provided for through subsequent generations after the modifying applications have been discontinued.

The alternative to the Lamarckian hypothesis of use and disuse and parallel induction is undoubtedly the neo-Darwinian proposition that unspecified and insubordinated chromosomal changes and gene mutations supply the basic material variations. These, then, through hybridity, ecological selection and perhaps other means, determine evolutionary advancement, *status quo*, deterioration or extinction of species and biocenoses. Though far from completeness, this appears by wide agreement to be the most valid answer yet available to the inquiry concerning the causes of evolution. The vast, consistent, critically experimental data and logical inferences which have accrued mainly during the past

¹ "Essays on Philosophical Biology," p. 49.

² "Recent Advances in Cytology," p. 483.

forty years seem to be overwhelmingly acceptable as favorable to the neo-Darwinian conception and opposed to any scheme involving the Lamarckian hypothesis.

Among biologists, Lamarckism has become almost bereft of candid advocates. Each recent effort to give it experimental, or any sort of acceptable, support and restitution to respectability has brought contumelies upon the vindicator. As a scientific tenet it seems to be as dead as the dodo. Nevertheless, belief in and reliance upon the main features of this doctrine continue widely among husbandmen, parents, old-wives, medicine men and a few scientists. The ardent hope and ecstatic anticipation that specifically adaptive effects of the environment upon the soma may be demonstrated to endure somehow among the progenies for indefinite numbers of generations after the affecting agencies have ceased to operate holds on with persistent obstinacy. So inveigling and compelling have been the wish and hope of this supposition that its seductive allurements have impaired or obfuscated the equanimity of ratiocination of a number of philosophical paleontologists, psychologists and biologists.

Nevertheless, and granting that its intrinsic scientific merits are in question, or entirely untenable, Lamarckism, among other things, undoubtedly has validity and authority as a tradition, folk-lore or article of faith and hope. Although belonging properly in the realm of phantasmal science, this tenet may be no less potent than romance, creative fiction, constructive imagination, wishful projection or teleology. Probably to no one, or combination, of these, except possibly teleology, has sentient man taken recourse more congruously and efficaciously than to the folk-lore tradition of use and disuse and the inheritance of the characteristics thereby acquired. In such ways this popular belief has undoubtedly exerted effective influence in husbandry, eugenics, supplication, importunity, sagacious planning, and in most all affairs of man during his long, hard march up the rocky road of progress. In this sense the subject has been previously broached.³

Although it is assumed that not one iota of scientific evidence has been marshalled in support of the supposition, phenomena do occur in nature and among domesticated plants, animals and man which present something of the *semblance or appearance* of Lamarckism. Indeed, it does appear as if there is a tendency for the more permanent, heritable and adaptive morphological struc-

³ AM. NAT., 61: 531-538, November-December, 1927.

tures and physiological properties to be closely parallel with, or accentuations of, the specifically environmentally induced somatic characteristics and functions, or *vice versa*. How do they become apparently so?

Mutations manifestly occur without specificity and particular reference to the surroundings, and completely insubordinate to the structures and functions which they condition whether they have adaptive capacities or not. The vast majority of all mutations are deleterious to the organisms in which they occur. Comparatively little account of these can be taken in nature because of their transitoriness and the lack of trustworthy records. They experience all degrees of survival from none at all to varying short lengths of time. Traces of them have been found in paleontology, some records have been made in the field, and the fact of their frequency amply established in many laboratories. Such unfavorable mutants are commonly ignored or forgotten also in the domesticated animals and man where the available records are often inadequate and undependable. Therefore, deleterious mutations are possibly of little, if any, general importance in evolution.

Other mutants, and perhaps in large numbers, are those that chance to conform somewhat better to the respective environs in which they occur. Most of these allow their bearers merely and miserably to suffer their living conditions and thus linger on the fringes of endurance and toleration. Indeed, it is likely that every organism at best experiences something of the element of mere toleration at times in, if not throughout, its life cycle. Examples could be cited by the hundreds, beginning with the turkey buzzard's and other carrion eaters' hard endurance, never enjoyment, of the ptomaines and other poisons in the putrefying flesh upon which they mainly subsist, and on to the most favorably conditioned bird or mammal. There are, then, just as indefinite numbers of mutants that happen better to adjust the individual organisms and species to their conglomerates of surroundings and relationships. Though perhaps fewer in numbers and still far from perfection, they are the ones that endure over the legions of those that are completely unfit and those that merely tolerate the environments for which they chance to be unaptly and precariously adapted. Under a given stress a mutant might be found to be beneficial, and in another complex of surroundings, to produce quite the opposite effect. There is, no

doubt, a shuttling back and forth in the value to a species of given mutants, depending upon the changes in the surroundings. Valuable mutants might logically be expected to spread rapidly and widely throughout a species, and harmful ones be limited, if they get any hold at all. Therefore, the few favorable ones should counteract the influence of a host of those that are not so propitious.

Somatic responses to changes in the surroundings exhibit extraordinary ranges of adaptive potency. Most organisms could not exist at all unless they were able to make somatic or physiological conformity to vast, frequent and often quite sudden changes in their environments. Such abilities are undoubtedly also products of mutations and selection. At no time must the features of the environment be so severe and inclusive as to surpass the abilities for adaptive somatic and aggregative responses of all the individuals in the widest range of the species. Among several habitats of grouse locusts which have been under observation for thirty years, not one has been fully available all the time. Most of them have been, due to drought, flood, oil, salinity or for some unobvious reason (possibly fungi or other disease), entirely unavailable one or more times. Fortunately, all of them have not been untenable at any one time. In connection with hybridity, the shifting of habitats from conditions of tenability to untenability and *vice versa*, over short and long stretches of time, gives almost any sort of mutant its chance, even though some of them may survive for only very short periods of time.

Now, if a sufficient number of individuals of a species can barely tolerate, through bodily and biocenotic responses, the strain imposed upon them by the progressively changing elements of the environment, times should come when chance mutations conforming with the nature of the somatic responses with which the organisms are already precariously meeting the stress will save the situation. If an environment changes gradually toward greater moisture, aridity, heat or cold or any conceivable complex, it may be logically expected that by relying upon the rare, fortuitously favoring mutants the species and aggregations may proceed in tolerably well-adapted relationship and parallel with it. Thus, tenaciously holding on by means of somatic variability and adaptability, remaining largely unaffected by the numerous inept, and profiting effectively by the apt, mutations, it is conceivable that individuals and populations may be directed specifi-

cally along the paths of, and parallel to, their gradually changing environments.⁴ Paleontology is filled with incidents of such occurrences as well as with contingencies with which such mutations as occurred could not cope.

Furthermore, organisms, plants more than animals, themselves contribute to the changes in their surroundings, as has been observed for an indefinitely long time and lately confirmed by the ecologists. If the changes have extended trends and directions, and the stress is not too severe for the existing possibilities of somatic and aggregative responses, the rare, fortuitously favorable mutants, which are likely some times to occur, enable the organisms further and better to tolerate, and adapt themselves to, the very changes for which they are in some way or another responsible. These operations may be so subtle and complex that without rigid scrutiny it may *appear as though* the organisms are consciously directing their own destinies, or that some transcendental power, or entelechy, is involved.

If the foregoing inferences are cogent there is, then, merely the *masquerade, the semblance or appearance*, undoubtedly a legitimate, efficacious and beneficent folk-lore, but *not the actuality, of Lamarckism*. The concept of orthogenesis which has engaged the attention of evolutionists from Eimer to Osborn, and the support of some of them, has been closely allied with Lamarckism. The explanation of either of these doctrines need not be different from the other. A complex of environment which changes gradually in a consistent direction would carry, *by the processes herewith suggested*, the fauna and flora conformably along with it. Thus, there may be also the *appearance or semblance*, likewise congruent and justifiable, *but not the reality of orthogenesis*.

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THE EFFECTS OF THE INJECTION OF ANTUITRIN G UPON A STRAIN OF HARELIP MICE

PHENOTYPICALLY normal individuals may show up (Normal Overlaps) in genetically harelip mice, as shown by Dr. Sheldon

⁴ Since this paper was presented to the local seminar, February 15, 1939, there has appeared in the Quarterly Review of Biology, 14: 1, pp. 65-67 (March, 1939), Dr. G. F. Gause's review of "The Organism as a Whole," etc., by Professor I. I. Schmalhausen. I have been pleased and encouraged to note that the author had apparently, according to the review, already presented possibly a somewhat analogous view.

C. Reed, of McGill University. It seemed that the percentage of these Normal Overlaps might be influenced by internal environment or physiological actions. Accordingly it was planned to try a number of different physiological agents in an attempt to reduce the frequency of harelip, or at least to change its frequency, by increasing or decreasing the frequency of Normal Overlaps. Since harelip embryologically is due to the failure of the palatine and maxillary processes to grow and fuse together in a normal way, it was decided to try Antuitrin G, a growth hormone, first.

Harelip albino mice were obtained from Dr. Reed. Some of these were injected intraperitoneally with a daily dose of $\frac{1}{3}$ rat unit, and some with $\frac{1}{10}$ rat unit of Antuitrin G in .5 cc of normal saline. These doses were started just before mating and continued for ten days. This covers the period necessary for the maxillary and palatine processes to grow together and fuse normally. The controls were not injected in the harelip strain; however, non-harelip strains were also used as controls, and these had .5 cc of normal saline injected. Mice shown in Table 1 were used first as controls, and later as experimental animals, so that genetic variability was reduced. A standard ration was used and identical conditions maintained in so far as was possible.

The results of the injection of Antuitrin G into harelip mice is shown in Table 1. A dose of $\frac{1}{3}$ rat units (R. U.) of Antuitrin G had no effect on the frequency of harelip when its 29.4 per cent. was compared with the 30.2 per cent. of the control; however, the dose of $\frac{1}{10}$ rat units seemed to increase the percentage of harelip as shown by the 16.5 per cent. increase. It would

TABLE 1
EFFECTS OF THE INJECTION OF ANTUITRIN G ON A STRAIN OF
HARELIP ALBINO MICE

	No. litters	No. indi- viduals	No. hare- lip	Per cent. hare- lip	Mean litter size	Mean indi- vidual weight
Control	13	63	19	30.2	4.84 \pm .60	1.33 \pm .065
Experimental 1/10 R. U. . .	5	30	14	46.7	6.00 \pm .76	1.11 \pm .068
Experimental 1/5 R. U. . .	4	17	5	29.4	4.25 \pm .43	1.27 \pm .048

seem that this dose caused many possible Normal Overlaps to give phenotypic expression to their genetic make-up. Inconclusive figures seem to suggest that a dose of $\frac{1}{3}$ rat units decreases the

litter size, while a dose of $\frac{1}{10}$ rat units increases the litter size as compared with the control. The individual weights of the new-born mice is lowered in each case by injections, and is lowered most by the $\frac{1}{10}$ rat unit dose. Dr. Reed has shown that large litters have an increased percentage of harelip and the figures given in Table 1 also suggest a correlation in this respect.

There was no clear-cut change in the percentage of the different types of harelip (dextral, sinistral or bilateral types), although there is a suggestion that the bilateral type and left type may be increased relative to the right type by the injections of Antuitrin G.

Three experimental females had double parturition with more young being born in one, two and four days following the first-born. Many premature mice also resulted from these injections. Trials on non-harelip strains of mice produced neither of these effects. The gestation time was reduced in the experimental mice to an average of 17.3 days, with a range of 16 to 19 days.

These strains of harelip mice have been lost, and since the author would like to continue this experiment and try many other physiological agents, he would appreciate it very much if any one who might have harelip and cleft palate show up in his colony would get in touch with him. This condition showed up originally in the Bagg albino strain.

Acknowledgment is given to students who have worked on this problem under the direction of the author: Frank Fuller, Ronald H. MacDonald, Harold Doyle, Robert Woods and Lyle Hill.

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THE DECREASE IN WEIGHT AND MORTALITY IN DEXTRAL AND SINISTRAL INDIVIDUALS OF THE SNAIL, *FRUTICICOLA LANTZI*

THE majority of species of terrestrial and watery snails are dextrally coiled. The recessive sinistral individuals are relatively rare. It is generally believed that dextrality or sinistrality of coiling is not associated with any physiological advantage or disadvantage of the individual bearing it. There are some preliminary suggestions, however, concerning the unequal fitness of both types. On the one hand, the sinistral mutants usually occur in small isolated colonies, often separated by a natural barrier from the area of the basic dextral form (Ludwig 1932, 1936).

The distribution of sinistral specimens over larger territories appears to be impeded by their ecologic weakness. On the other hand, some physiological observations suggest that while in the individual of the typical coil the steric configuration of organic substances participating in the determination of twist might well conform to the levorotatory steric configuration of the other constituents of protoplasm, some enzymatic discoordinations might be expected in the organisms of the inverted individual (Gause, 1939). The purpose of this investigation is to compare under controlled laboratory conditions some physiological characteristics in the dextral and sinistral individuals of the same species of snail.

The terrestrial mollusk *Fruticicola lantzi* was collected in an isolated mountainous colony in the region of Alma-Ata (Middle

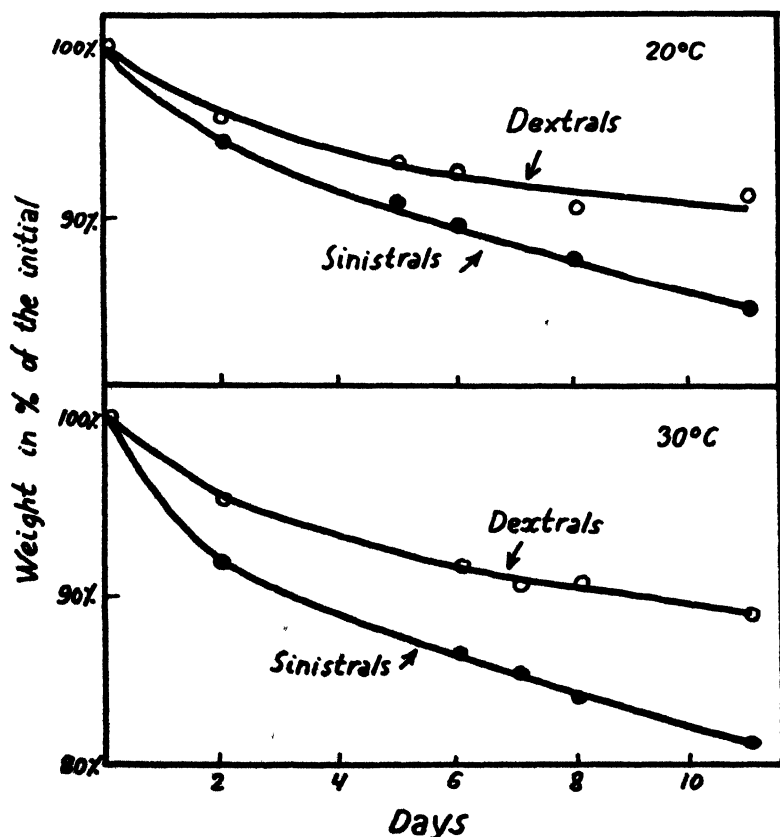


FIG. 1. The average curves of the decrease in wet weight in starving young snails *Fruticicola lantzi* at different temperatures.

Asia). Both dextral and sinistral individuals occurred together at the time of observation, and were apparently bred under perfectly identical geographic and ecologic conditions. The material was not easily obtainable, and we studied altogether 300 individuals of both types in the work extending over a period of two years.

The snails, obtained by parcel post from Middle Asia in a state of estivation (into which they very easily lapse in case of deficiency of moisture), were placed for reviving into a big moist chamber, and during several days before the experiment were fed on carrots. Then the snails were numbered and weighed individually on the analytical balance. Before every weighing each snail was carefully wiped with absorbent cotton.

In two series of experiments the decrease in wet weight in dextral and sinistral starving individuals was studied. The first series of experiments was conducted with young snails (diameter of the shell 10–11 mm). Ten dextral and ten sinistral individuals were placed in each moist glass chamber, in which there was nothing but a small amount of water. In all experiments snails were daily transferred into a new clean chamber. One chamber

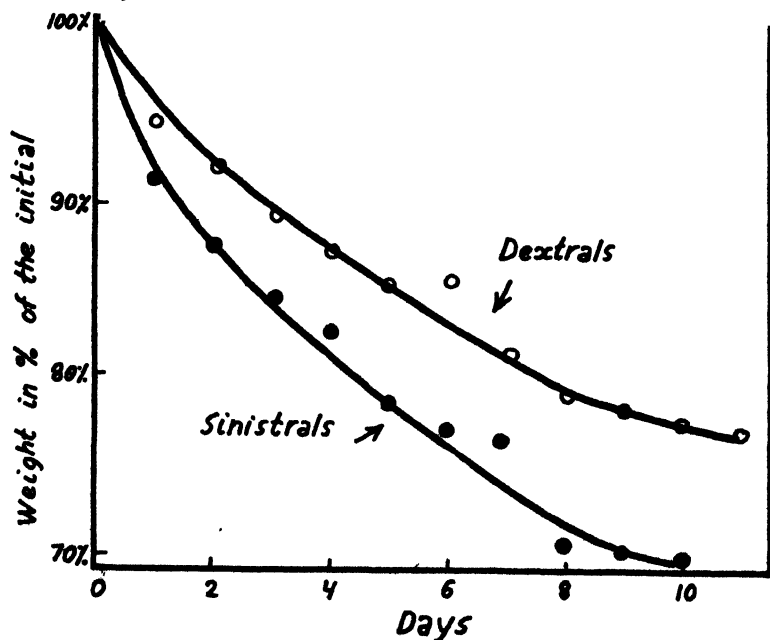


FIG. 2. The average curves of the decrease in wet weight in starving adult snails *Fruticicola lanesti* at 18° C.

was kept at 20° C. and another at 30° C. The change of wet weight of snails in these experiments represented a smooth gradual decrease. Fig. 1 shows that at both temperatures sinistral individuals lose in wet weight more rapidly than the typical dextral forms.

In the second series of experiments the decrease in wet weight in the starving adult snails (diameter of the shell 18–19 mm) in big moist chambers was studied. Twenty six dextral and twenty six sinistral individuals were kept under observation. Before death the enfeebled starving snail usually can not retreat into its shell, and such snails were no longer weighed. Fig. 2 makes it evident that the sinistrally twisted starving individuals decrease in wet weight more rapidly than the dextral snails.

In order to eliminate possible fluctuations in water content of the snails, the previous data collected in the summer of 1938 were extended in 1939 by measurements of the decrease in the dry weight in starving adult snails of the fresh collection. Both dextral and sinistral snails were combined into pairs of identi-

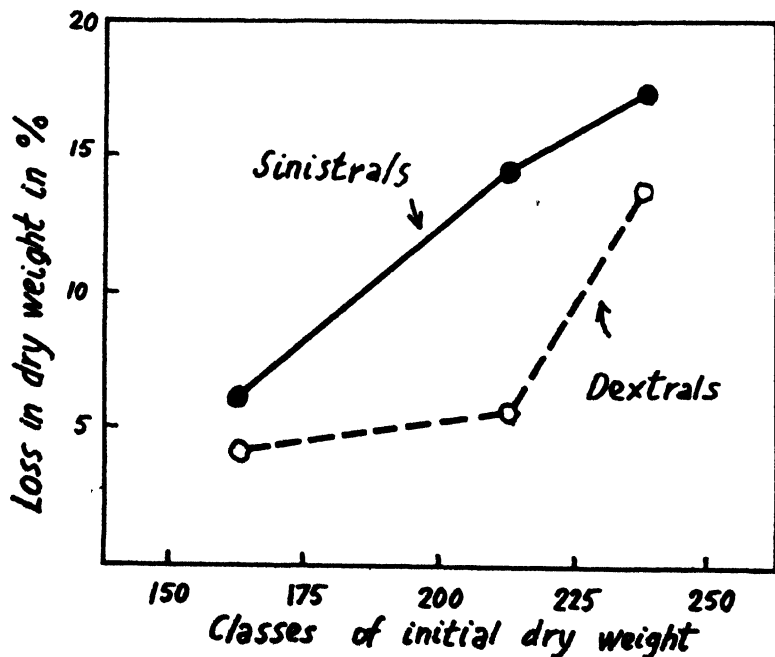


FIG. 3. The decrease in dry weight in dextral and sinistral starving adult snails *Fruticicola lanati* belonging to different classes of initial dry weight in milligrams.

cally twisted specimens of approximately equal wet weight. The dry weight of one member of the pair was immediately determined, while the dry weight of another member was measured after a five-days starvation in a moist chamber at 25° C., and these two weights were later compared. For measurement of the dry weight the shell of the snail was carefully eliminated, and the remaining body was dried at from 70 to 75° C. for eight hours. Seventy four dextral and forty eight sinistral individuals were used in this investigation. Fig. 3 shows that sinistral individuals of all initial weight classes lose in the dry weight much more rapidly than the dextral individuals of the same initial weight classes.

The records of mortality in populations of starving snails have shown that in sinistrally twisted forms the mortality rate exceeds that of the dextrally twisted ones. In the first experiment with fifty dextral and fifty sinistral specimens, the mortality on the fifth day attained 27 per cent. and 40 per cent. and in the second experiment 42 per cent. and 54 per cent. correspondingly. As the sinistral snails for the maintenance of their living burn up per unit of time a comparatively larger amount of food reserves, they consequently expend their available food resources more quickly and die off more rapidly than the dextrally twisted forms of the same species.

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INDEX

NAMES OF CONTRIBUTORS ARE PRINTED IN SMALL CAPITALS

- Allometry, Evolutionary, in Skeleton of Domesticated Dog, H. LUMER, 439
- Animals, Stenothermy and Eurythermy of, in Relation to Habitat, J. A. MOORE, 188
- Antuitrin G, Effects of the Injection of, upon a strain of Harelip Mice, L. C. GLASS, 566
- Anura, Observations on the Ecology and Natural History of, A. N. BRAGG, 322, 424
- BAITSELL, G. A., Modern Concept of the Cell as a Structural Unit, 5
- BALAMUTH, W., Contributions to the Problem of Regeneration in Protozoa, 528
- BEASLEY, J. O., Origin of American Tetraploid *Gossypium* Species, 285
- BLOOM, W., Local and Generalized Defense Reactions in Animals, 129
- BRAGG, A. N., Observations on the Ecology and Natural History of Anura, 322, 424
- BUCHANAN, J. W., Introduction, Symposium on Regeneration, 481
- BUCHHOLZ, J. T., and M. KAEISER, A Statistical Study of Two Variables in the Sequoias, 279
- Cancer, Studies on the Chemotherapy of, in Mice Derived from Genetic Principles, L. C. STRONG, 180
- Cell, Theory: Modern Concept as a Structural Unit, G. A. BAITSELL, 5; Present Status of Mitosis, F. SCHRADER, 25; Problem of Cell Individuality in Development, P. WEISS, 34; What of the Future?, C. E. MCCLUNG, 47; Reactions, I. F. LEWIS, 97
- CHATTERS, R. M., Induction by Fast Neutrons of Mutations in *Antirrhinum*, 283
- CHILD, C. M., Social Integration as a Biological Process, 389
- Chromosomes, Sex, Questionable Existence in Angiosperms, H. W. JENSEN, 67; Translocation between Sperm and Egg, A. R. SIDKY, 475
- COLE, L. J., Introduction, Symposia on Speciation, 193
- COPPELAND, E. B., A Biologist's Appreciation of Religion as a Factor in Social Evolution, 418
- COWLES, R. B., Reptilian Sensitivity to High Temperatures, 542
- Craspedacusta Again, A. M. REESE, 180
- CUMLEY, R. W., and M. R. IRWIN, Speciation from the Point of View of Genetics, 222
- CURTIS, W. C., Histologic Basis of Regeneration and Reassociation in Lower Invertebrates, 487
- DELBRÜCK, M., Radiation and the Hereditary Mechanism, 350
- DICE, L. R., Ecologic and Genetic Variability within Species of *Peromyscus*, 212; Speciation in *Peromyscus*, 289
- DOBZHANSKY, TH., Speciation as a Stage in Evolutionary Divergence, 312
- Drosophila*, hydei and virilis, Subspecies, Hybrids and Speciation in, W. P. SPENCER, 157; melanogaster, Longevity in, D. GREIFF, 363
- Dugesia Tigrina, Reproduction of, R. KENK, 471
- Egg, Abnormal Shape of, H. M. SCOTT, 185
- Fowl, Crossing Over and Sex in, D. C. WARREN, 93; Breast Ridge in Domestic, S. S. MUNRO and I. L. KOSIN, 382
- Fowls, White Wyandotte, Relation between Breed Characteristics and Poor Reproduction in, F. B. HUTT, 148
- Frogs, Adaptative Differences in Egg Membranes of, J. A. MOORE, 89
- GAUSE, G. F., and N. P. SMARAGDOVA, Decrease in Weight and Mortality in Dextral and Sinistral Individuals of the Snail, 568
- Genes, and Chromosome Aberrations, Symbolising of, Correspondent, 287
- GLASS, L. C., Effects of the Injection of Antuitrin G upon a Strain of Harelip Mice, 566
- Gossypium*, Species, Origin of American Tetraploid, J. O. BEASLEY, 285
- GREIFF, D., Longevity in *Drosophila melanogaster*, 363

- Habrobracon, *Brevicornis*, Occurrence of Diploid Males in, B. R. SPEICHER and K. G. SPEICHER, 379; Do Females Sting Their Eggs?, A. R. WHITING, 468
- HOLMES, S. J., Introduction, Symposium on the Biological Basis of Social Problems, 385; Ethics of Enmity in Social Evolution, 409
- HUBBS, C. L., Speciation of Fishes, 198
- HUTT, F. B., Relation between Breed Characteristics and Poor Reproduction in White Wyandotte Fowls, 148
- HYMAN, L. H., Aspects of Regeneration in Annelids, 513
- IRWIN, M. R., and R. W. CUMLEY, Speciation from the Point of View of Genetics, 222
- JENSEN, H. W., Questionable Existence of Sex Chromosomes in Angiosperms, 67
- KAEISER, M., and J. T. BUCHHOLZ, A Statistical Study of Two Variables in the Sequoias, 279
- KENK, R., Reproduction of *Dugesia Tigrina*, 471
- KOSIN, I. L., and S. S. MUNRO, Breast Ridge in Domestic Fowl, 382
- Lamarckism, Masquerade of, R. K. NABOURS, 562
- LEWIS, I. F., Cell Reactions, 97
- LUMER, H., Evolutionary Allometry in the Skeleton of the Domesticated Dog, 439
- McCLUNG, C. E., Cell Theory, What of the Future?, 47
- MAYR, E., Speciation Phenomena in Birds, 249
- Mechanisms, Defense, in Plants and Animals: Local Reactions in Plants, F. W. WENT, 107; Generalized Defense Reactions in Plants, W. C. PRICE, 117; Local and Generalized Defense Reactions in Animals, W. BLOOM, 129
- Medusa, Fresh-Water, Another New York Record for, R. A. VAN AUKEN, 95
- MOORE, J. A., Adaptative Differences in Egg Membranes of Frogs, 89; Stenothermy and Eurythermy of Animals in Relation to Habitat, 188
- MUNRO, S. S., and I. L. KOSIN, Breast Ridge in Domestic Fowl, 382
- Mutations, in *Antirrhinum*, Induction by Fast Neutrons of, R. M. CHATTERS, 283
- NABOURS, R. K., Masquerade of Lamarckism, 562
- Naturalists, American Society of, 97-148
- Polyploidy, Significance of, in Plant Evolution, G. L. STEBBINS, JR., 54
- PRICE, W. C., Generalized Defense Reactions in Plants, 117
- Radiation and the Hereditary Mechanism, M. DELBRÜCK, 350
- REESE, A. M., Craspedacusta Again, 180
- Regeneration: Introduction, J. W. BUCHANAN, 481; Histologic Basis of, and Reassociation in Lower Invertebrates, W. C. CURTIS, 487; Environmental Control of, in Euplanaria, O. RULON, 501; Aspects of, in Annelids, L. H. HYMAN, 513; Contributions to the Problem of, in Protozoa, W. BALAMUTH, 528
- Reptilian Sensitivity to High Temperatures, R. B. COWLES, 542
- RITTER, W. E., A Few Words about Nature, 386
- RULON, O., Environmental Control of Regeneration in Euplanaria, 501
- SCHRADER, F., Present Status of Mitosis, 25
- SCOTT, H. M., Abnormal Shape of Egg, 185
- Sequoias, A Statistical Study of Two Variables in, J. T. BUCHHOLZ and M. KAEISER, 279
- Sex-Linkage in *Pteromalus*, P. W. WHITING, 377
- SIDKY, A. R., Translocation between Sperm and Egg Chromosomes, 475
- SMARAGDOVA, N. P., and G. F. GAUSE, Decrease in Weight and Mortality in Dextral and Sinistral Individuals of the Snail, 568
- Snail, Decrease in Weight and Mortality in Dextral and Sinistral Individuals of, G. F. GAUSE and N. P. SMARAGDOVA, 568
- Social Problems, Biological Basis of: Introduction, S. J. HOLMES, 385; A Few Words about Nature, W. E. RITTER, 386; Social Integration as

- a Biological Process, C. M. CHILD, 389; Naturalist as a Social Phenomenon, F. B. SUMNER, 398; Ethics of Enmity in Social Evolution, S. J. HOLMES, 409; A Biologist's Appreciation of Religion as a Factor in Social Evolution, E. B. COPELAND, 418
- Speciation, and the Relation of Genetics to Geographical Distribution: Introduction: L. J. COLE, 193; of Fishes, C. L. HUBBS, 198; Ecologic and Genetic Variability within Species of *Peromyscus*, L. R. DICE, 212; from the Point of View of Genetics, M. R. IRWIN and R. W. CUMLEY, 222; Breeding Structure of Populations in Relation to, S. WRIGHT, 232; Phenomena in Birds, E. MAYR, 249; in *Peromyscus*, L. R. DICE, 289; Levels of Divergence in *Drosophila*, W. P. SPENCER, 299; as a Stage in Evolutionary Divergence, TH. DOBZHANSKY, 312
- SPEICHER, B. R., and K. G. SPEICHER, Occurrence of Diploid Males in *Habrobracon Brevicornis*, 379
- SPEICHER, K. G., and B. R. SPEICHER, Occurrence of Diploid Males in *Habrobracon Brevicornis*, 379
- SPENCER, W. P., Subspecies, Hybrids and Speciation in *Drosophila hydei* and *virilis*, 157; Levels of Divergence in *Drosophila* Speciation, 299
- STEBBINS, G. L., JR., Significance of Polyploidy in Plant Evolution, 54
- STRONG, L. C., Studies on the Chemotherapy of Cancer in Mice Derived from Genetic Principles, 180
- SUMNER, F. B., Naturalist as a Social Phenomenon, 398
- VAN AUKEN, R. A., Another New York Record for Fresh-Water Medusa, 95
- WARREN, D. C., Crossing Over and Sex in Fowl, 93
- WEISS, P., Problem of Cell Individuality in Development, 34
- WENT, F. W., Local Reactions in Plants, 107
- WHITING, A. R., Do *Habrobracon* Females Sting Their Eggs?, 468
- WHITING, P. W., Sex-Linkage in *Pteromalus*, 377
- WRIGHT, S., Breeding Structure of Populations in Relation to Speciation, 232

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